The Diagnosis and Classification of Lymphoproliferative Disorders in Ghana

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Medicine by Dr Elizabeth Angela Stephens.

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Table of contents

Abstract

Purpose of project

The purpose of the project was to set up a system to diagnose and categorise lymphoproliferative disorders in Ghana using modern diagnostic techniques, not currently available locally in a clinically meaningful way. Results would be used to inform treatment decisions, as well as provide epidemiological data regarding lymphoproliferative disorders in West Africa. This system would be robust and sustainable so that local clinicians could continue to use it once the initial project had finished and, as resources became available locally, transfer of skills and techniques was envisaged so that they could be performed locally.

Methods and Results

Biopsy samples from patients with a suspected lymphoproliferative disorder, from two teaching hospitals in Ghana, were sent by post to the Haematological Malignant Diagnostic Service, Leeds and results were e-mailed back to clinicians in Ghana. This system was feasible, sample quality was preserved and adequate for processing and turnaround times were rapid enough for results to be used to guide patient management. Using this model, 61 patients with lymphoproliferative disorders were diagnosed and classified according to the World Health Organisation classification of lymphoproliferative disorders. Preliminary epidemiological data regarding lymphoproliferative disorders in West Africa was also collected with a view to expanding on this in future studies. The system was robust and is potentially sustainable.

There were significant differences between the spectrum of lymphoproliferative disorders diagnosed in Ghana and those diagnosed at HMDS. In the project there were significantly more cases of splenic marginal zone lymphoma, peripheral T-cell lymphoma and T-cell acute lymphoblastic leukaemia, but less cases of diffuse large B-cell lymphoma than seen

at the Haematological Malignant Diagnostic Service. Amongst the patients with splenic marginal zone lymphoma there was a striking excess of female patients. We envisaged that the excess numbers of cases of splenic marginal zone lymphoma seen in Ghana compared to Yorkshire would be due to chronic infection causing antigenic stimulation. We did not see an excess of cases with mutated immunoglobulin heavy chain genes and in those cases that were mutated, the mutation load was relatively low.

This project suggests that there are fascinating differences between the spectrum of lymphoproliferative disorders in temperate and tropical climates and has highlighted potential areas of further research that could provide important insights into the biology of lymphoproliferative disorders.

List of Abbreviations

ABVD	Doxorubicin, bleomycin, vinblastine and dacarbazine
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
ATLL	Adult T-cell leukaemia/lymphoma
AWLP	All Wales Lymphoma Panel
BL	Burkitt lymphoma
BMA	Bone marrow aspirate
BSH	British Society of Haematology
CHOP	Cyclophosphamide, doxorubicin, vincristine, prednisolone
CLL	Chronic lymphocytic leukaemia
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
CTCL	Cutaneous T-cell lymphoma
Dif	Differential
DLBL	Diffuse large B-cell lymphoma
DNA	Deoxyribonucleic acid nucleic acid
EBV	Epstein Barr virus
EDTA	Ethylenediamine tetra-acetic acid
EFS	Event free survival
ELS-I	El Salvador I
ESR	Erythrocyte sedimentation rate
FBC	Full blood count
FIM	Fatal infectious mononucleosis
FISH	Fluorescence *in-situ* hybridisation
FITC	Fluorescein isothiocyanate
FL	Follicular lymphoma
FNA	Fine needle aspirate
H&E	Haematoxylin and eosin

HAART	Highly active antiretroviral therapy
HCL	Hairy cell leukaemia
HCV	Hepatitis C virus
HHV6	Human herpes virus 6
HHV8	Human herpes virus 8
HIV	Human immunodeficiency virus
HL	Hodgkin lymphoma
HMDS	Haematological malignant diagnostic service
HMS	Hyper-reactive malarial splenomegaly
HTLV-1	Human T-cell leukaemia virus type 1
Ig	Immunoglobulin
IGHV	Immunoglobulin heavy chain variable
ILSG	International Lymphoma Study Group
IPI	International prognostic index
IPSID	Immunoproliferative small intestinal disease
JSS	Junior secondary school
KATH	Komfo Anokye Teaching Hospital
KBTH	Korle Bu Teaching Hospital
KNUST	Kwame Nkrumah University of Science and Technology
KSHV	Kaposi Sarcoma herpes virus
KW	Kruskal Wallis
LD	Lymphocyte depleted
LDH	Lactate dehydrogenase
LMP-1	Latent membrane protein-1
LP	Lymphocyte predominate
LPD	Lymphoproliferative disorder
LSTM	Liverpool School of Tropical Medicine
M:F	Male:Female
MALT lymphoma	Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue
MC	Mixed cellularity

MCL	Mantle cell lymphoma
MGUS	Monoclonal gammopathy of undetermined significance
MRI	Magnetic resonance imaging
MZL	Marginal zone lymphoma
NH_4Cl	Ammonium chloride
NHL	Non-Hodgkin lymphoma
NK	Natural killer
NMIMR	Noguchi Memorial Institute for Medical Research
NPC	Nasopharyngeal carcinoma
NS	Nodular sclerosing
NSAIDs	Non-steroidal anti-inflammatory drugs
PAL	Pyothorax-associated lymphoma
PB	Peripheral blood
PBS	Phosphate buffered solution
PCBCL	Primary cutaneous B-cell lymphoma
PCR	Polymerase chain reaction
PCS	Pearson Chi-Square
PE	Phycoerythrin
PEL	Primary effusion lymphoma
PET	Positive emissions tomography
PID	Primary immunodeficiency disorder
PTCL	Peripheral T-cell lymphoma
PTLD	Post-transplant lymphoproliferative disorder
REAL classification	Revised European-American Classification of Lymphoid Neoplasms
SHML	Sinus histiocytosis with massive lymphadenopathy
SJCRH	St Jude's Children's Research Hospital, Memphis
SLE	Systemic lupus erythematosus
SLVL	Splenic lymphoma with villous lymphocytes
SMZL	Splenic marginal zone lymphoma
SND	Splenomegaly non-diagnostic marrow

SSS	Senior secondary school
TCR	T cell receptor
TSL	Tropical splenic lymphoma
UPS	United Postal Service
UR	Unrecordable
US	Ultrasound
WCC	White cell count
WHO	World Health Organisation

Introduction

Purpose and overview of the project

The purpose of the project was to set up a system to diagnose and categorise lymphoproliferative disorders (LPDs) in Ghana using modern diagnostic techniques, not currently available locally, in a clinically meaningful way. Results would be used to inform treatment decisions, as well as provide epidemiological data regarding LPDs in West Africa. This system would be robust and sustainable so that local clinicians could continue to use it once the initial project had finished and, as resources became available locally, transfer of skills and techniques was envisaged so that they could be performed locally.

Biopsy samples from patients with a suspected LPD, from two teaching hospitals in Ghana, were sent by post to the Haematological Malignant Diagnostic Service (HMDS), Leeds and results were e-mailed back to clinicians in Ghana. This system was feasible, sample quality was preserved and adequate for processing and turnaround times were rapid enough for results to be used to guide patient management. Using this model, 61 patients with LPDs were diagnosed classified according to the World Health Organisation (WHO) classification of LPDs (Jaffe *et al*, 2001).

Brief Background

LPDs, cancers of the lymphoid system, occur throughout the world (Parkin *et al*, 1999). Much is known about LPDs in developed countries, for example incidence, immunophenotype and genotype, but in comparison there is little published information about LPDs in developing countries (Jaffe *et al*, 2001). There is marked variation in the incidence and sub-types of LPDs worldwide (Jaffe *et al*, 2001). As will be discussed in Chapter 1, there is strong evidence for a link between LPDs and infection and this, along with variations in race and socioeconomic status may account for much of the global

variation, however the influence of other factors such as poverty and nutrition is not known.

The increasing incidence, morbidity, mortality and socioeconomic impact of chronic illnesses, such as hypertension, diabetes, stroke and cancer, in developing countries has become a major concern (Jones, 1999; Jones *et* al, 2006; Kanavos, 2006; Mellstedt, 2006; Ngoma, 2006; Abegunde *et al*, 2007). Access to, and availability of, healthcare in many developing countries has improved with a resultant increase in availability of both chemotherapy and radiotherapy. It is therefore crucial that diagnostic techniques necessary to diagnose cancers, such as LPDs, in developing countries, improve so that the correct treatment is given.

Work to investigate patients with massive splenomegaly in Kumasi, Ghana previously suggested the presence of a LPD, Tropical Splenic Lymphoma (TSL), which appeared to share some similarities with hyper-reactive malarial splenomegaly (HMS) suggesting the possibility of malaria as an aetiological factor in the development of TSL (Bates & Bedu-Addo, 1997a). Further work was limited by a lack of locally available diagnostic techniques and difficulty transporting fresh samples to the UK for further investigation. Recent improvements in diagnostic techniques available in the UK, as well as improved communication systems, mean that remote investigation of patients with a suspected LPD on stored tissue has become feasible.

Due to the clear variations in LPDs between developing and developed countries it is not possible to extrapolate our knowledge and treatment modalities from developed to developing countries. Information regarding the epidemiology and pathogenesis of LPDs in developing countries may also help our understanding of lymphomagenesis which may in turn improve the treatment of LPDs throughout the world. LPDs in developing countries therefore need to be investigated as a matter of urgency.

Dr Stephens' interest and role in the project

Dr Stephens spent a two month medical student elective period at Bawku District Hospital in North-East Ghana in 1997. Later, as a third year haematology specialist registrar on the Leeds rotation, she considered a career in tropical haematology. Collaboration between HMDS, the Liverpool School of Tropical Medicine (LSTM) and the two teaching hospitals in Ghana was being discussed. Dr Stephens expressed an interest in becoming involved in the collaborative project and registered the project with Liverpool University as an MD project.

As will be discussed in Chapter 2, Dr Stephens played a key role in planning, obtaining ethical approval for, setting up, undertaking and writing up the project. For the duration of the project Dr Stephens lived in Kumasi, Ghana where she entered patients into the project and worked as both a haematology specialist and as a lecturer. She also spent time in Accra, entering patients there, as well as time at HMDS and LSTM.

Structure of the thesis

The thesis is divided into 7 Chapters and there are 9 Appendices. In the first chapter the background to the project is set out and the relevant published literature is reviewed. The second chapter describes how the remote diagnostic service was set up and discusses some of the challenges that were met in doing so. In the third chapter the characteristics and diagnoses of the patients that were entered into the project are described. The fourth chapter focuses on those patients that were diagnosed with a LPD and compares the spectrum of LPDs diagnosed in Ghana to the spectrum of LPDs diagnosed at HMDS and then divides the LPD diagnoses into 4 groups and discusses them in greater detail. In the fifth chapter, 5 interesting cases are described and discussed and in the sixth chapter the project is summarised and discussed including limitations of the project, potential pitfalls in the diagnosis of lymphomas, important issues relating to research in developing countries and future research potentials.

Chapter 1 - Literature Review and Background Information

Introduction

This chapter presents information regarding health services in Ghana, LPDs in general and in developing countries and finally models that exist for collaborative diagnosis between developed and developing countries. The relevant literature is reviewed to provide a background to, and put in context, the work carried out in the project.

Background information about health service in Ghana

Geography and Demographics

Ghana is on the coast of West Africa bordered on the remaining three sides by francophone countries – Côte d'Ivoire, Togo and Burkina Faso. (Figure 1.1) Ghana, formerly the Gold Coast, was the first African colony to gain independence from colonial rule in 1957.

Figure 1.1 – Map of Ghana (www.ashantiafricantours.com)

The national statistics are fairly impressive for a developing country (Table 1.1), but there are large economic divides between the relatively affluent cities and the poor rural regions, especially the northern regions. This is also reflected in inequalities in health care provision, for example in 2001 the entire Northern region (population 1.9 million, area 70 000km^2) had 17 doctors compared to approximately 500 doctors in Greater Accra (population 4 million) (Horton, 2001). There are also variations in disease prevalence other than those due to local environmental factors. The national Human Immunodeficiency Virus (HIV) prevalence in 2004 was 3.6%, but prevalence rates above 5% were recorded in the Central and Eastern regions in the same year (2005 Programme of Work, www.ghanahealthservice.org).

Table 1.1 - Ghana: a development summary with UK comparisons (Horton, 2001; Human Development Report, 2001)

	Ghana	**UK**
Population (millions)	18.9	59
Fertility rate	4.6	1.7
Life expectancy (yrs)	56.6	77.5
Adult literacy rate (%)	29.7	
School enrolment (%)	29.7	100
Health spending (% gross domestic product)	1.8	5.9
Health spending per capita ($)	85	1532
Physicians/100 000 people	6	164
Infant mortality rate (per 1 000 live births)	63	6
Adequate sanitation (%)	63	100
Births with skilled staff (%)	44	100
Malaria cases/100 000	11 941	0
Tuberculosis cases/100 000	53	10
Adults with HIV (%)	3.6	0.11

Structure of the health care system

There are two main teaching hospitals in Ghana - Korle Bu Teaching Hospital (KBTH) in Accra and Komfo Anokye Teaching Hospital (KATH) in Kumasi. A third medical school was started in 1996 at the Northern Regional Hospital in Tamale. It does not, however, have the facilities to teach beyond the pre-clinical years and has been beset by difficulties since it was built. There are a further 8 regional government hospitals, 91 district hospitals (62 government, 29 mission) and 124 other hospitals. Ghana also has 558 health centres, 1085 clinics and 320 maternity homes – these are a mixture of government, mission and private run.

Access to and payment for health care

Following Independence from British rule the government introduced an almost fee free healthcare system. In 1969 fees were reintroduced and in 1985 the 'fee for service' system was introduced. This system charged a set fee for services, for example an out patient review or operation. A slight fall in clinic attendances was noticed at this time, but this became more pronounced in the early 1990s with the introduction of the 'cash and carry' system (Biritwum, 1994). In the cash and carry system all items, including drugs, needles and syringes, had to be paid for at the point of delivery. A deposit was taken from patients on admission to hospital to try to avoid delays in initial health care, but a study at KATH found that despite this, patients did not get the necessary initial investigations or treatment (Appiah-Poku, 1996). Certain groups of patients, for example those with tuberculosis or leprosy, were exempt from paying fees, but this was rarely implemented (Nyonator *et al*, 2001). A national health insurance system was called for by these authors and this was finally in place in most of the country by January 2006, although it had been introduced in some rural areas during 2005.

The national health insurance scheme requires people to register and pay an annual fee. This means tested fee is between 74 000 and 180 000 cedis per person per year (£4.65-£11.25). Each person is issued with a card and this entitles them to free health care. Certain illnesses and procedures are not included, for example treatment for some

malignancies. The fees per procedure have risen significantly with the introduction of the national insurance system, for example the fee for the administration of intravenous chemotherapy has risen from 50 000 to 100 000 cedis. It is hoped that the national insurance system will encourage patients to seek medical care earlier in their illness and that this will improve outcome.

In order to truly improve access to health care however it needs to be more evenly distributed throughout the country especially in rural areas. The Ghana Health Sector 2005 Programme of Work notes that from 1997 to 2003 outpatient services increased from 0.36 to 0.55 per capita (www.ghanahealthservice.org). This is a large increase, but reflects both an increase in the number of people attending and the number of times each attended and does not just reflect increased access to health care. Patients can be referred to teaching hospitals from clinics and hospitals throughout the country, but due to lack of referral, lack of money, transportation difficulties etc only a very small proportion of the population access tertiary level health care. There are no figures available, however as the reasons are so complex.

The diagnosis and treatment of malignancies in Ghana

The diagnosis and treatment of malignancies in developing countries is becoming more important as therapeutic modalities become available and many of the previous causes of morbidity and mortality such as malnutrition are addressed (Greenberg *et al,* 2001; Mellstedt 2006). KBTH has a fully functioning department of pathology, where samples are processed and reported and pathologists trained, thus facilitating the diagnosis of all malignancies. KBTH has also had a radiotherapy department since 1997. Until 2004, when the current radiotherapy centre opened at KATH, this was the only fully functioning radiotherapy centre in the country – the nearest centers were in Nigeria to the east and Senegal to the west so patients came from the whole of Ghana, as well neighbouring countries, to KBTH for radiotherapy.

At KATH the pathology department was unable to process and report histology specimens for many years including the 15 months of this project. This hindered the

diagnosis and treatment of malignancies as samples had to be sent out to private laboratories with the associated extra cost to patients, delay in results and potential concerns regarding quality of results. Until 2004 oncology clinics were held in the old radiotherapy building where a limited amount of radiotherapy was also given. Haematology clinics were held in the office of one of the specialists and patients were referred to Accra for most radiotherapy. The new radiotherapy/oncology centre was opened in 2004 – this included clinic rooms for haematology and oncology clinics as well as a CT scanner and the new radiotherapy equipment.

LPDs in General

Definition of LPDs

LPDs, cancers of the lymphoid system, are a heterogeneous group of neoplastic disorders of the immune system including lymphomas (non-Hodgkin and Hodgkin), myeloma and lymphoid leukaemias.

Diagnosis of LPDs

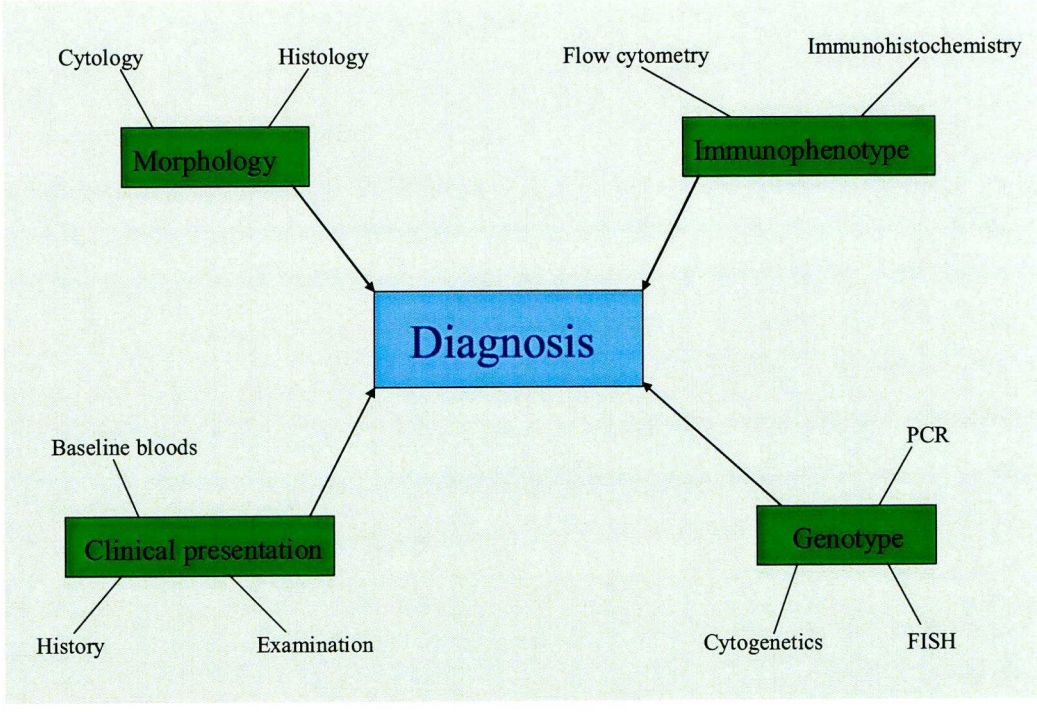
The diagnosis of LPDs in developed countries has evolved as diagnostic techniques have improved. Previously LPDs were described morphologically, but now immunophenotyping and genotyping are part of routine diagnosis. As a result there is now a multi-faceted approach to the diagnosis of LPDs in developed countries which includes the clinical presentation, morphological features, immunophenotype and genotype. (Figure 1.2) This has been incorporated in the evolution of classification systems for LPDs as will be discussed later. Diagnostic techniques are mentioned briefly below and described in greater detail in Appendix 1.

Morphology

Morphology remains crucial in the diagnosis of LPDs. Stained peripheral blood (PB), bone marrow aspirate (BMA) and fine needle aspirate (FNA) slides allow an assessment of cell cytology. Cytology can confirm the probable diagnosis in some LPDs such as chronic lymphocytic leukaemia (CLL), acute lymphoblastic leukaemia (ALL) and

myeloma. These morphological diagnoses can be confirmed by immunophenotyping as discussed below. In order to make a definitive diagnosis of most lymphomas, however, histology of a biopsy specimen is necessary as this allows architectural features as well as cytological features to be assessed. (Appendix 1)

Figure 1.2 - The diagnosis of LPDs



Immunophenotyping

Immunophenotyping is the demonstration of antigens, expressed by cells, using labeled monoclonal antibodies. Different techniques can be used to demonstrate both surface antigen and cytoplasmic antigen. In recent years there has been a rapid increase in the number of monoclonal antibodies available for diagnostic purposes as well as an increase in knowledge base regarding the patterns of antigens expressed both on normal lymphocytes and LPDs. Immunophenotyping adds a great deal of information to basic morphology in the diagnosis of haematological malignancies, especially in the diagnosis of LPDs.

Flow cytometry allows rapid immunophenotyping of fresh samples for example PB, BMA, cerebrospinal fluid (CSF), serous effusions and lymph node biopsies. Samples are prepared by cell lysis or density gradient centrifugation and then conjugated with a panel of antibodies labelled with a fluorescent dye. The fluorescent characteristics of the cells are then assessed using a flow cytometer. (Appendix 1)

Immunocytochemistry and immunohistochemistry have advanced so much in recent years that it is now possible to use a much wider range of antibodies to immunophenotype cytology preparations and fixed samples. This has several advantages over flow cytometry – including the ability to immunophenotype samples that cannot be transported to the laboratory within 24-48 hours, archive material and samples that have been processed in other laboratories. (Appendix 1)

Molecular techniques

In conventional cytogenetics, stained chromosomes from metaphase preparations are examined. Samples must be sent fresh to the laboratory and transported in preservative free heparin. In many indolent or low-grade LPDs it is difficult to obtain enough cells in metaphase for conventional cytogenetics and so this technique is of limited value in the diagnosis of LPDs. (Appendix 1)

Fluorescence *in-situ* hybridisation (FISH) allows genetic translocations, deletions and additions to be identified with deoxyribonucleic acid nucleic acid (DNA) probes. Probes are fluorochrome-bound oligonucleotides, labeled with chromosomes, or parts of chromosomes, which are complementary to specific sequences on a chromosome. FISH allows cytogenetic analysis to be performed on fresh, fixed or stored tissues. Both interphase (non-dividing) and metaphase (dividing) preparations are suitable. This has several advantages over conventional cytogenetics; firstly cell culture medium and rapid analysis are not required facilitating retrospective diagnosis, secondly it is applicable to indolent LPDs as cells do not need to be in metaphase. (Appendix 1)

Polymerase chain reaction (PCR) can be used in LPD diagnosis to detect certain chromosomal abnormalities and demonstrate clonality. PCR is performed on DNA and so can be performed retrospectively if cells are frozen at the time of diagnosis. (Appendix 1)

As part of the normal differentiation process B-cells undergo rearrangement of their immunoglobulin (Ig) genes and T-cells undergo rearrangement of their T-cell receptor (TCR) genes. Clonality can therefore be demonstrated by PCR analysis of the Ig and TCR genes of B and T-cell LPDs respectively. Analysis of the immunoglobulin heavy chain variable (*IGHV*) region of a B-cell clone allows an assessment of whether the cell of origin has undergone somatic hypermutation in the germinal centre and provides evidence of antigen-driven clonal selection (Kuppers *et al*, 1999). This will be discussed further in Chapter 4.

Imaging

As imaging techniques have improved they have taken on a crucial role in the diagnosis and staging of LPDs. At present computed tomography (CT) scans are routinely used to stage both Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL), as well as to assess nodal and extra-nodal disease in plasmacytomas, ALL and CLL. The advances in CT scanning results have meant that laparoscopic staging procedures are no longer necessary, nor are diagnostic splenectomies in HL. Plain x-rays are of particular use in myeloma to look for lytic bone lesions. Magnetic resonance imaging (MRI) is more sensitive than plain x-rays at demonstrating bone disease and can also be useful when investigating LPDs involving the central nervous system (CNS).

Ultrasound (US) scans are useful to demonstrate nodes in the neck and assess intra-abdominal disease, especially the liver, spleen and kidneys. US scans cannot be used to assess retroperitoneal disease, however. Positive emissions tomography (PET) scanning is becoming more readily available in developed countries and is likely to play an important role in both staging disease and assessing response to treatment, especially where there is a residual lesion post chemotherapy.

Classification of LPDs

LPD classification systems have evolved over the years as diagnostic techniques have improved and LPDs have become better understood. Previously lymphoid leukaemias were classified separately to lymphomas and there were separate classification systems for HL and NHL. As understanding of LPDs has improved it has been recognised that these illnesses form a spectrum of disease and so they have been incorporated into the same classification system, firstly in the Revised European-American Classification of Lymphoid Neoplasms (REAL classification), published in 1994 and later in the World Health Organisation (WHO) Classification of Tumours of the Haematopoietic and Lymphoid Tissues (Harris *et al*, 1994; Jaffe *et al*, 2001).

The REAL classification was the result of a meeting of the International Lymphoma Study Group (ILSG), a group of 19 expert haematopathologists from North America, Asia and Europe, in Berlin in 1993. It uses all available information – morphology, immunophenotype, genetic features and clinical features to define a disease entity. (Figure 1.2) Since its publication the REAL classification has been subjected to rigorous review to verify its reproducibility and clinical relevance.

The WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues is the result of a collaborative project between the European Association for Haematopathology and the Society for Haematopathology. Work on the WHO classification started in 1995 and was published as a book in 2001 (Jaffe *et al*, 2001). The WHO classification of NHL is based on the REAL classification, but extends the principles of disease definition and consensus building to myeloid, mast cell and histiocytic neoplasms. The development of the WHO classification involved a Clinical Advisory Committee made up of expert haematologists and oncologists to advise the pathologists on clinical aspects of the classification system. The result is a comprehensive, internationally accepted and clinically relevant classification system incorporating all haematological neoplasms that will be subject to continual assessment and revision as new techniques and information become available.

The WHO classification recognises three major categories of lymphoid neoplasms: B-cell neoplasms, T- and Natural Killer (NK)-cell neoplasms and HL. (Appendix 3) Both solid and circulating phases are present in many lymphoid neoplasms therefore lymphomas and lymphoid leukaemias are classified together. For example B-cell CLL and B-cell small lymphocytic lymphoma are different manifestations of the same neoplasm, as are lymphoblastic lymphomas and lymphoblastic leukaemias.

Lymphomagenesis and the genetic changes underlying LPDs

As more is understood about the normal development and function of lymphocytes, so our understanding of lymphomagenesis has improved. Lymphoid precursor cells, derived from bone marrow stem cells, undergo gene rearrangement in the bone marrow and become naïve B-cells that express surface Ig. Naïve B-cells leave the bone marrow and circulate in blood though lymph nodes until they encounter antigen and undergo blast transformation. They then migrate to the centre of a primary follicle, the germinal centre; they are now termed centroblasts. Here they undergo class switching and somatic hypermutation and mature to become centrocytes. This results in cells that produce high affinity IgG or IgA antibodies. Cells that do not achieve this die through apoptosis. Centrocytes leave the germinal centre and become memory B-cells and then antibody producing plasma cells.

This scheme of B-cell ontogeny can be correlated with the cell of origin of the B-LPDs: lymphoid precursor cells correspond with acute lymphoblastic leukaemia/lymphoma, naïve B-cells with CLL and Mantle cell lymphoma (MCL), germinal centre B-cells with HL, Burkitt lymphoma (BL), Follicular lymphoma (FL), Diffuse large B-cell lymphoma (DLBL), memory B-cells with marginal zone lymphoma (MZL) and plasma cells with myeloma. Class switching and hypermutation are inherently risky; this is why the germinal centre is the source of so many B-cell LPDs. Genetic changes can be translocations, deletions or additions of chromosomes. Translocations involving the *IGHV* locus at 14q32 are critical in lymphomagenesis. (Table 1.2)

Table 1.2 – Translocations involving the *IGHV* locus resulting in LPDs

Translocation	Abnormality	Associated LPD
t(14;18)	bcl-2	FL, DLBL
t(11;14)	cyclin D1	MCL, Myeloma
t(8;14)	c-MYC	BL, transformed FL
t(3;14)	bcl-6	DLBL
t(9;14)	Pax5	MZL, DLBL, myeloma
t(14:19)	bcl-3	MZL, CLL
t(4;14)	Fgfr3	myeloma
t(14;16)	CMAF	myeloma
t(3;14)	Fox P1	DLBL, MZL

Translocations causing chimeric fusion genes also result in LPDs such as t(11;18) resulting in API2-MALT1 in MZL, t(2;5) resulting in NPM-ALK in anaplastic large cell lymphoma and t(9;22), t(12;21), t(4;11) and t(1;19) in ALL. Other translocations can also result in LPDs such as those involving bcl6 in DLBL. Deletions (-) and additions (+) of chromosomes are also associated with LPDs. (Table 1.3)

Table 1.3 – Numerical chromosome changes resulting in LPDs

Numerical change	Associated LPD
-13q, -11q, -17p	CLL
-13, -13q	myeloma
-6q	Waldestroms macroglobulinaemia
+3, +18	MZL
Hyperdiploidy	ALL, myeloma
+12	CLL

Geographical variation in LPDs and underlying genetic changes

There are marked geographical variations in the incidence and spectrum of both clinical and sub-clinical LPDs, in particular mature B-cell, T-cell and NK-cell neoplasms (Anderson *et al*, 1998; Parkin *et al*, 1999; Jaffe *et al*, 2001; Weisenburger *et al*, 2005).

Mature B-cell neoplasms are more common in developed countries than in less developed countries – the annual incidence of NHL in males ranges from 15.61/100 000 in North America to 1.28/100 000 in China with intermediate rates elsewhere (Parkin *et al*,1999). There is also significant geographical variation in the relative frequency of different types of B-cell neoplasms, for example, FL is more common in developed countries and is uncommon in South America, Eastern Europe, Africa and Asia (Anderson *et al*, 1998; Jaffe *et al*, 2001). BL, in contrast, is endemic in equatorial Africa, but accounts for only 1-2% of lymphomas in the United States and Western Europe (Jaffe *et al*, 2001). Nasal and nasal-type NK/T-cell lymphomas and aggressive NK/T-cell leukaemia are much more common in Asians than they are in other races (Anderson *et al*, 1998).

There are also geographical variations in the genetic changes underlying LPDs, for example the t(14;18) translocation in FL. Biagi and Seymour (2002) found that although up to 50% of healthy subjects in Asia and the West have bcl-2 rearrangements, the incidence of bcl-2 rearrangements in FL in Asian populations was significantly lower than in the West. Shiramizu *et al* (1991) looked at chromosomal breakpoints in endemic versus sporadic BL and their relation to Epstein Barr virus (EBV). They found that c-MYC breakpoints differ significantly in endemic versus sporadic BL. Understanding more about geographical variations in the genetic changes underlying LPDs and the impact of variations in breakpoints will aid our understanding of lymphomagenesis.

Links between LPDs and infections

There are established links between certain LPDs and infections. (Table 1.4) Infectious agents can cause LPDs in 1 of 3 ways; either by directly transforming lymphocytes (eg EBV) or by causing immunosuppression (eg HIV) or by chronic immune stimulation (eg Hepatitis C virus (HCV) and certain bacteria) (Engels, 2007).

EBV Infection

An association between infectious mononucleosis and HL was first recognised in the 1950s, before EBV had been discovered (Ambinder, 2007). The link between EBV and

endemic BL was described a decade later (Epstein *et al*, 1964). Subsequent work has shown that EBV is associated with other LPDs, as well as several non-LPD neoplasms including: nasopharyngeal carcinoma (NPC), some gastric carcinomas, foregut lymphoepitheliomas, inflammatory pseudotumours of the liver and spleen and HIV-associated smooth muscle neoplasms (Rezk & Weiss, 2007).

Following acute EBV infection the virus enters a latent phase in circulating B-cells. 3 types of EBV latency have been described – type 1 (the latency programme) in which only 2 latent genes are expressed, type 2 (the default programme) in which a restricted array of latent genes are expressed and type 3 (the growth programme) in which all 9 latent genes are expressed (Thorley-Lawson, 2001; Heslop, 2005). It is thought that this complex mechanism helps EBV-infected cells to evade the immune system.

These latency types also correlate with the three groups of EBV-associated LPDs (Heslop, 2005; Rezk & Weiss, 2007). First BL, which can occur in both immunocompromised and immunocompetent patients, tends to express type 1 latency. Second, EBV-associated NHLs that occur in immunocompetent patients and EBV-associated HL tend to express type 2 latency (Kapatai & Murray, 2007). Finally, EBV-associated NHLs that occur in immunocompromised patients tend to express type 3 latency – this group will be discussed later in the chapter.

The WHO classification divides BL into endemic, sporadic and immunodeficiency-associated subtypes (Jaffe *et* al, 2001). EBV is demonstrable by in situ hybridisation in almost all endemic BL cases in African children, 15-20% of sporadic BL and 30- 40% of HIV-associated BL (Prevot *et al*, 1992; Hamilton-Dutoit *et al*, 1993; Young & Rickinson, 2004). Translocation of the c-MYC gene underlies all types of BL; however the breakpoints occur at different positions in the different forms of BL probably due to variations in pathogenesis (Brady *et al*, 2007).

Elevated antibody titres to EBV in patients with HL were first described in 1971 (Levine *et al*, 1971). Since then, further work has shown that EBV is frequently associated with

classical HL, especially mixed-cellularity (MC) HL, but rarely with nodular lymphocyte predominant HL (Pallesen *et* al, 1991; Chan, 1999). EBV-associated HL appears to be related to immunocompetence - there is a higher incidence of EBV-positive cases in developing countries and HIV-positive patients (Leoncini *et* al, 1996; Glaser *et al*, 2003). There is also a bimodal age-distribution with EBV-positive cases being more common in the young and the old, where the immune system is underdeveloped or ageing, and less common in adolescents, where the immune system is more robust (Jarrett *et al*, 1991).

The effect of highly active anti-retroviral therapy (HAART) on the incidence of EBV-positive HIV-associated HL is complex with the incidence being greatest with moderate immunosuppression and reducing when CD4 counts fall further or recover (Ambinder, 2007; Grogg *et al*; 2007). The reason for this is unclear; however the inflammatory response appears to play an important role in the pathogenesis of virus-associated HL.

Aggressive NK-cell leukaemia/lymphoma, extranodal NK/T-cell lymphoma, T-cell rich B-cell lymphoma, angioimmunoblastic lymphoma, pyothorax-associated lymphoma (PAL) and some cases of DLBL and CD30 positive anaplastic large cell lymphoma are EBV-associated NHLs seen in immunocompetent hosts (Sasajima et al, 1993; Jaffe et al, 2001; Young & Rickinson, 2004; Heslop, 2005).

Chronic active EBV infection occurs in apparently immunocompetent individuals and predisposes to the development of haemophagocytic syndromes, organ dysfunction and lymphoma (Kimura *et al*, 2001). There is an abnormal immune response to EBV infection resulting in a high viral load with fever, hepatosplenomegaly and pancytopenia as well as a high associated mortality.

Other viral infections

HIV-associated LPDs occur due to immunosupression as opposed to the virus itself, as will be discussed later.

Kaposi sarcoma herpes virus (KSHV), also named human herpes virus 8 (HHV8), is closely related to EBV and is also lymphotrophic. KSHV/HHV8 is consistently identified

in primary effusion lymphoma (PEL), multicentric Castleman's disease associated plasmablastic lymphoma and multicentric Castleman's disease – three HIV-associated disorders (Soulier *et al*, 1995; Carbone *et al*, 1996; Dupin *et al*, 2000). Cases of KSHV/HHV8 positive solid lymphomas have also been reported in both HIV positive and negative patients (Carbone *et al*, 2005a).

Human T-cell leukaemia virus type 1 (HTLV-1), a deltaretrovirus, is linked to the development of adult T-cell leukaemia/lymphoma (ATLL) (Jarrett, 2006). The risk of developing ATLL amongst HTLV-1 infected individuals is approximately 4% and there is a long latency period between infection and development of ATLL, suggesting that other factors are also involved (Shuh & Beilke, 2005).

HCV is lymphotrophic as well as being hepatotrophic and is associated with the development of type II mixed cryoglobulinaemia, an autoimmune disorder that, in about 10% of patients, may evolve into a malignant lymphoma (Zignego *et al*, 1997; Misiani *et al*, 1992; Agnello *et al*, 1992; Ferri *et al*, 1993; De Re *et al*, 2000).

HCV appears to be linked to B-cell LPDs - one American and two Italian groups showed a significantly increased incidence of hepatitis C infection in unselected patients with B-cell LPDs versus local controls, however two British groups showed no increase in hepatitis C infection in patients with B-cell LPDs (Ferri *et al*, 1994; Silvestri *et al*, 1996; Hanley *et al*, 1996; McColl & Tait, 1996; Zuckerman *et al*, 1997). The difference may reflect the relatively small numbers that were included, especially in the British groups, and geographical and/or ethnic variation - of note 78% of the patients in the American study were Hispanic. Gisbert *et al* (2003) performed a meta-analysis and review of 48 studies evaluating the prevalence of HCV in B-cell NHL; they found a prevalence of approximately 15% in patients with B-cell NHL, higher than the general population (1.5%) and patients with other haematological malignancies (2.9%). Again there appeared to be a marked geographical variation in these numbers.

Despite the fact that HCV is lymphotrophic, LPD development related to HCV seems to be indirect probably due to chronic antigenic stimulation (Jarrett, 2006; De Re *et al*, 2000). HCV is mainly associated with low-grade B-cell lymphomas –FL, MZL, extranodal MZL of mucosa-associated lymphoid tissue (MALT lymphoma), although it has been reported in association with most subtypes of both B- and T-cell NHL (Engels *et al*, 2004). Hermine *et al* (2002) treated nine patients with both splenic MZL (SMZL) and hepatitis C with interferon alone or in combination with ribavirin and demonstrated a complete remission in eight patients and partial remission in the ninth patient.

A potential link between simian virus 40, a macaque polyomavirus, and LPDs has been the subject of some debate. Polio vaccines administered to an estimated 150 million people were inadvertently contaminated with simian virus 40 between 1955 and 1963. Some groups have shown evidence for a link (Martini *et al*, 1998; Vilchez *et al*, 2002; Shivapurkar *et al*, 2002; Nakatsuka *et al*, 2003; Vilchez *et al*, 2005). Others have shown no evidence for such a link (MacKenzie *et al*, 2003; Capello *et al*, 2003; Brousset *et al*, 2004; Thu *et al*, 2006).

Measles and human herpes virus 6 (HHV6) have also been linked with HL although there is conflicting evidence regarding the latter (Benharroch *et al*, 2004; Cartwright & Watkins 2004). Finally van den Bosch suggests that arboviruses are likely cofactors for the development of endemic BL, along with EBV and plant extracts, with malaria only playing a small part; the reasons for this are mentioned later (van den Bosch, 2004).

Bacterial Infections

The response to bacterial infections has been implicated in the aetiology of several types of MALT lymphoma (Jaffe, 2004; Parsonnet & Issacson, 2004; Guidboni *et al*, 2006). The link between gastric MALT lymphoma and Helicobacter pylori infection is the best studied of these. T-cells activated by H. pylori antigens are necessary for the continued proliferation of gastric MALT lymphoma cells (Hussell *et al*, 1993). Interestingly, immunoglobulins produced by gastric MALT lymphoma cells appear to recognise autoantigens, as opposed to H. pylori derived antigens, suggesting that perhaps H. pylori antigens act as 'molecular mimickers' to trigger autoimmune reactivity (Qin *et al*, 1995;

Negrini *et al*, 1996; Guidboni *et al*, 2006). Eradication of H. pylori with antibiotic therapy has been shown to lead to remission of the lymphoma (Wotherspoon *et al*, 1993). De Sanjose *et al* (2004) found that H. pylori was associated with a 3-fold excess risk of splenic marginal zone lymphoma. H.pylori has also been implicated in ocular adnexa MALT-type lymphomas (Ferreri *et al*, 2006).

Helicobacter heilmannii-associated primary gastric low-grade MALT lymphoma showed a complete response to a standard H. pylori eradication regimen in five patients that were H. pylori negative by culture, histology, serology and PCR (Morgner *et al*, 2000).

Chlamydia species are obligate intracellular bacteria that grow in eukaryotic cells and tend to cause persistent infections – they therefore have the potential to cause chronic antigenic stimulation resulting in tumour development (Guidboni *et al*, 2006). Chlamydia psittaci has also been implicated in ocular adnexal MZL. Ferreri *et al* (2004) found that 32 out of 40 ocular adnexal lymphoma biopsies were positive for C.psittaci DNA compared to 0 out of 20 non-neoplastic biopsies and 3 out of 26 reactive lymph node biopsies. 9 out of 21 patients with C.psittaci positive lymphomas had C.psittaci in their PB mononuclear cells compared to 0 out of 38 healthy individuals. They treated 7 patients with doxycycline and showed that C.psittaci DNA was not detectable after three weeks of treatment in all 7; only 4 of these patients had a measurable mass and a response was demonstrated in 2 of these. These results have not been reproduced in other centres however (Daibata *et al*, 2005; Vargas *et al*, 2006; Rosado *et al*, 2006; Chanudet *et al*, 2006; Mulder *et al*, 2006; De Cremoux *et al*, 2006).

Chlamydia pneumoniae has been implicated in cutaneous T-cell lymphoma (CTCL) (Abrams *et al*, 1999 and 2001). Following treatment for CTCL cases became less positive for C. pneumoniae and the authors hypothesise that chronic infection with C. pneumoniae leads to expansion of C. pneumoniae-specific T-cells, potentiating the development of cutaneous lymphoma. Rossler *et al* (2003) were unable to replicate these findings in 24 patients with mycosis fungoides and so further work is needed to investigate the potential link between C. pneumoniae and CTCL.

Several Borrelia burgdorferi species have been associated with, and Borrelia DNA found in, cases of primary cutaneous B-cell lymphoma (PCBCL) in Europe although response to antibiotic therapy has been variable (Cerroni *et al*, 1997; Kutting *et al*, 1997; Jelic & Filipovic-Ljeskovic, 1999; Goodlad *et al*, 2000; Slater, 2001; de la Fouchardiere *et al*, 2003). Interestingly no Borrelia DNA was found in 24 cases of cutaneous MZL from Asia and 34 cases from the USA although Borrelia DNA was identified in control samples in the latter study; this is thought to be related to the distribution and type of Borrelia present in these areas (Li *et al*, 2003; Wood *et al*, 2001).

Immunoproliferative small intestinal disease (IPSID), also known as alpha chain disease, is particularly common in the Mediterranean basin, the Middle East, the Far East and North Africa (Lecuit *et al*, 2004). IPSID is a small intestinal MALT-lymphoma that is associated with the production of truncated α heavy chain proteins and tends to occur mostly in older children and young adults of low socio-economic status (Al-Saleem & Al-Mondhiry, 2005). IPSID shows excellent response to antibiotics if treated in the early stages of disease (Akbulut *et al*, 1997). Although patients with IPSID tend to have multi-bacterial overgrowth in their intestines, the pathogen responsible for the development of IPSID appears to be Campylobacter jejuni (Lecuit *et al*, 2004). Some doubt remains as C. jejuni, unlike bacteria implicated in other forms of MALT lymphoma, is not a persistent coloniser in humans and C. jejuni is very common, whereas IPSID is rare and only occurs in endemic areas (Parsonnet & Issacson, 2004; Guidboni *et al*, 2006).

PAL is a B-cell NHL that develops in the pleural cavity of patients following a long history (>20 years) of pyothorax resulting from previous artificial pneumothorax as treatment for tuberculosis (Aozasa *et al*, 2005). PAL is also strongly associated with EBV infection and most cases have been reported in Japan, where artificial pneumothorax was a relatively common treatment for tuberculosis (Sasajima *et al*, 1993; Aozasa *et al*, 2005). Askling & Ekbom (2001) also report an increased incidence of NHL amongst survivors of severe tuberculosis diagnosed prior to 1953 and propose that this may be a factor in the increased incidence of NHL that has been seen in recent years.

Oscier *et al* (2002) report a case of MALT-lymphoma of the bladder that macroscopically responded to antibiotics for chronic urinary tract infection. This highlights the need for further work looking into chronic antigenic stimulation in LPDs at various sites and with various etiologic agents.

Protozoal Infections

Links between malaria and LPD have been postulated. In particular malaria appears to play a part in the development of endemic BL (Facer & Playfair, 1989). This has, however been disputed by van den Bosch (2004) who argues that if early EBV infection and malaria were the only prerequisites for endemic BL it would be far more common than it is and that arbovirus infection as a cofactor would explain the clustering and cyclical nature of cases of endemic BL, as well as the reduction in cases that follows malaria eradication programmes. A link between malaria infection and tropical splenic lymphoma has also been postulated as will be discussed later in this chapter (Bates & Bedu-Addo, 1997a).

Table 1.4 – Infections associated with various LPDs

Infection	Associated LPDs	References
Viruses - EBV	BL- Endemic, Sporadic and Immunodeficiency related, post-transplant and other immunodeficiency linked LPD, lymphomatoid granulomatosis, aggressive NK-cell leukaemia, nasal NK/T-cell lymphoma, HL, PAL	Epstein *et al*, 1964; Levine *et al*, 1971; Jarrett *et al*, 1991; Pallesen *et* al, 1991; Prevot *et al*, 1992; Hamilton-Dutoit *et al*, 1993; Sasajima *et al*, 1993; Leoncini *et* al, 1996; Chan, 1999; Jaffe *et al*, 2001; Kimura *et al*, 2001; Thorley-Lawson, 2001; Glaser *et al*, 2003; Young & Rickinson, 2004; Thorley-Lawson& Gross, 2004; Heslop, 2005; Ambinder, 2007; Brady *et al*, 2007; Grogg *et al*; 2007; Kapatai & Murray, 2007; Rezk & Weiss, 2007

Table 1.4 – Infections associated with various LPDs continued

Infection	Associated LPDs	References
KSHV/HHV8	PEL, multicentric Castleman's disease associated plasmablastic lymphoma, multicentric Castleman's diasease, solid lymphomas	Soulier *et al*, 1995; Carbone *et al*, 1996; Dupin *et al*, 2000; Carbone *et al*, 2005a
HTLV-1	ATLL	Jarrett, 2006; Shuh & Beilke, 2005
Simian virus 40	Various lymphomas	Martini *et al*, 1998; Vilchez *et al*, 2002; Shivapurkar *et al*, 2002; Nakatsuka *et al*, 2003; Vilchez *et al*, 2005; MacKenzie *et al*, 2003; Capello *et al*, 2003; Brousset *et al*, 2004; Thu *et al*, 2006
Measles	HL	Benharroch *et al*, 2004
HHV6	HL	Cartwright & Watkins, 2004
Arboviruses	Endemic BL	van den Bosch, 2004
Bacteria – H pylori	Gastric MALT lymphoma, SMZL, ocular adnexal MZL	Jaffe *et al*, 2004; Parsonnet & Issacson, 2004; Guidboni *et al*, 2006; Hussell *et al*, 1993; Qin *et al*, 1995; Negrini *et al*, 1996; Wotherspoon *et al*, 1993; de Sanjose *et al*, 2004; Ferreri *et al*, 2006
H.Heilmanni	Gastric MALT lymphoma	Morgner *et al*, 2000
Chlamydia psitacci	Ocular adnexal MZL	Guidboni *et al*, 2006; Ferreri *et al*, 2004; Daibata *et al*, 2005; Vargas *et al*, 2006; Rosado *et al*, 2006; Chanudet *et al*, 2006; Mulder *et al*, 2006; De Cremoux *et al*, 2006

Table 1.4 – Infections associated with various LPDs continued

Infection	Associated LPDs	References
Chlamydia pneumoniae	CTCL	Abrams *et al*, 1999; Abrams *et al*, 2001; Rossler *et al*, 2003
Borrelia Burgdorferi	Primary cutaneous B-cell lymphoma	Cerroni *et al*, 1997; Jelic & Filipovic-Ljeskovic 1999; Goodlad *et al*, 2000; Slater, 2001; de la Fouchardiere *et al*, 2003, Kutting *et al*, 1997; Li *et al*, 2003; Wood *et al*, 2001
Campylobacter jejuni	IPSID	Lecuit *et al*, 2004; Al-Saleem & Al-Mondhiry, 2005; Akbulut *et al*, 1997; Parsonnet & Issacson, 2004; Guidboni *et al* 2006
Mycobacterium tuberculosis	PAL, NHL	Aozasa *et al*, 2005; Sasajima *et al*, 1993; Askling & Ekbom, 2001
Unknown	MALT lymphoma of bladder	Oscier *et al*, 2002
Protozoa – Malaria	EndemicBL, Tropical splenic lymphoma	Facer & Playfair, 1989; Bates & Bedu-Addo, 1997a; van den Bosch, 2004

Links between LPDs and immunodeficiency

The WHO classification divides immunodeficiency-associated LPDs into 4 groups depending on the underlying immunodeficiency: 1) primary immunodeficiency syndromes and other primary immune disorders (PIDs); 2) HIV infection; 3) iatrogenic suppression in patients' post solid organ or bone marrow allografts; 4) iatrogenic immunosuppression with methotrexate, commonly in autoimmune disease (Jaffe *et al*, 2001). The underlying mechanism of lymphomagenesis in each of these groups varies slightly with resulting variation in the different LPDs seen, however they are commonly EBV or other oncovirus-related, extranodal (including a high incidence of CNS involvement) and aggressive LPDs.

LPDs and primary immunodeficiency syndromes and other PIDs

The immune defect varies slightly in the different PIDs, resulting in a heterogeneous group of LPDs (Jaffe *et al*, 2001, Kumar *et al*, 2006). (Table 1.5) PID-associated LPDs usually present in childhood, commonly with general malaise - fever and fatigue.

DLBL is the commonest LPD in PID; other LPDs that also occur in immunocompetent patients, including HL, are also seen. LPDs seen in the context of immunodeficiency also occur in PID including polymorphic lymphoproliferations resembling post-transplant LPD (PTLD), fatal infectious mononucleosis (FIM) and lymphomatoid granulomatosis (Jaffe *et al*, 2001). In ataxia telagiectasia T-cell LPDs are more common than B-cell LPDs (Taylor *et al*, 1996). T-cell LPDs can also occur in some of the other PIDs, although less frequently than their B-cell counterparts.

Table 1.5 – Mechanisms underlying LPD development in some PIDs (Jaffe *et al*, 2001, Kumar *et al*, 2006)

Primary immune disorder	**Mechanism underlying development of LPD**
X-linked lymphoproliferative syndrome (Duncan syndrome)	Defect in SH2D1A resulting in uncontrolled T-cell proliferation
Hyper IgM syndrome	Mutations in CD40 ligand affecting B-cell/T-cell interactions and effective B-cell differentiation
Ataxia telangectasia	Defective DNA repair due to *ATM* gene mutations
Autoimmune lymphoproliferative syndrome	Defective apoptosis due to *FAS* gene mutations
Nijmegen breakage syndrome	Defects in DNA repair
Common variable immunodeficiency	Chronic antigenic stimulation resulting lymphoid hyperplasia which may predispose to the development of lymphoid malignancy
Wiskott-Aldrich syndrome	Mutation of Wiskott-Aldrich Syndrome protein
Chediak-Higashi syndrome	Mutations in LYST (a lysosomal trafficking protein)

FIM occurs due to an uncontrolled systemic proliferation of EBV-positive B-cells which can result in potentially fatal haemophagocytosis (Jaffe *et al*, 2001). Lymphomatoid granulomatosis is an EBV-associated LPD that can occur in apparently immunocompetent individuals; however thorough investigation of these patients identifies an underlying immune defect commonly of T-cell function (Sordillo *et* al, 1982; Wilson *et al*, 1996).

LPDs and HIV infection

HIV-associated LPDs also fall into three categories (Jaffe *et al*, 2001). The first category includes LPDs that also occur in the absence of HIV infection – for example classical HL, BL, peripheral T-cell lymphoma (PTCL), DLBL with centroblastic/immunoblastic features (including primary cerebral lymphoma) and MALT lymphoma. The second category contains LPDs that are rare in the absence of HIV infection – PEL, plasmablastic lymphoma of the oral cavity and multi-centric Castleman disease. The third category covers LPDs that also occur in other immunodeficiency states – polymorphic B-cell lymphoma and lymphomatoid granulomatosis (Jaffe *et al*, 2001; Carbone *et al*, 2005b; Lim & Levine, 2005; Jarrett, 2006).

HIV-associated LPDs are thought to occur due to a combination of reduced immune surveillance of oncogenic viruses, such as EBV and HHV8, and the immunosuppression and cytokine dysregulation that occurs as a result of HIV infection (Grogg *et al*, 2007). The transforming properties of the HIV virus itself have been postulated, but HIV sequences have not been demonstrated in most HIV-associated LPDs although there is some in vitro evidence that HIV may in itself be oncogenic (Knowles, 2003; Grogg *et al*, 2007)

With the introduction of HAART the overall incidence of HIV-associated NHL in San Diego fell from 29.6 per 1000 person-years to 6.5 per 1000 person-years (Diamond *et al,* 2006). In the context of HIV infection centroblastic DLBL, BL and classical HL are associated with moderate immunosuppression whereas immunoblastic DLBL, primary cerebral lymphoma and PEL tend to occur later in the disease when CD4 counts are very low. With the introduction of HAART the relative proportions of the different HIV-

associated LPDs therefore changed with an increase in centroblastic DLBL (21% to 44%), BL (4% to 9%) and classical HL (in particular nodular sclerosing (NS) HL) and a reduction in immunoblastic DLBL (26% to 7%), primary cerebral lymphoma (28% to 17%) and PEL (Diamond *et al,* 2006; Grogg *et al*, 2007).

HIV-associated NHLs usually present with advanced systemic disease often involving extranodal sites (CNS, gastrointestinal tract, liver and bone marrow) and occasionally at sites that are rarely involved other than in the context of HIV-infection such as the anorectum and the heart (Grogg *et al*, 2007).

LPDS and iatrogenic suppression in patients post solid organ or bone marrow allografts

PTLDs are a heterogenous group of disorders ranging from polyclonal proliferations to aggressive lymphomas. They can be subdivided into 4 categories (Jaffe *et al*; 2001):
1) Early lesions – reactive plasmacytic hyperplasia, infectious mononucleosis-like;
2) Polymorphic PTLD;
3) Monomorphic PTLD – these are sub-classified according to the WHO classification and include DLBL, BL, myeloma, plasmacytomas and PTCL;
4) HL and HL-like PTLD.

PTLDs occur due to defective immune surveillance due to immunosuppression as well as chronic antigenic stimulation from the engrafted organ. Most post-transplant LPDs are EBV-related and early lesions and polymorphic PTLD often regress if the immunosuppression is reduced or stopped. The relative risk of PTLD following solid organ transplant varies from 240 post heart-lung transplant to 13 post kidney transplant (Opelz & Dohler, 2004). This variation probably reflects the level of immunosuppression used in the different transplant settings.

LPDs and iatrogenic immunosuppression with methotrexate

The risk of LPDs in patients treated with methotrexate is due to both immune suppression induced by the drug, and the increased risk of lymphomas in patients with autoimmune disorders which is discussed later. Methotrexate-associated LPDs are as heterogeneous as the other immunosuppression related LPDs and include DLBL, HL and tumours

resembling polymorphous PTLD (Jaffe *et al*, 2001). These disorders are commonly EBV-related and occasionally regress when methotrexate is stopped.

Other aetiological factors described in LPDs

Infection and immunosuppression are the best documented aetiological factors in LPDs. LPD incidence in the US rose by 3-4% per year from 1973 to the mid 1990s and a similar rise was seen in other developed countries (Fisher & Fisher, 2004). Approximately 50% of this increase is felt to be due to improved diagnosis and reporting, altered classification systems and HIV; however the other 50% is largely unexplained. This has prompted many authors to hunt for other potential aetiological factors to explain this rise with a view to developing new therapeutic and preventative strategies. In most cases of LPD however, an underlying cause cannot be identified.

Links between LPDs and ethnicity

Certain ethnic groups have been found to have an increased predisposition to certain diseases. These effects sometimes persist despite a change in environment for example when people emigrate from their homeland. This implies that there is an increased genetic susceptibility as opposed to environmental factors. Due to shifts in populations over the years it is now possible to study some ethnic or racial groups living in different environments to their original homelands and compare to other ethnic groups living in the same environment or the same ethnic group in the original environment. It is also possible to look at different ethnic groups living in the same environment where several indigenous groups have co-existed with minimal intergroup reproduction thus maintaining separate gene pools.

Lynch *et al* (2004) reviewed the available literature via a MedLine search and found that Ashkenazi Jews had a higher incidence of NHL and carcinomas of the ovary, pancreas and stomach, but lower incidence of carcinomas of the lung, penis and cervix. Several studies have looked at the incidence of CLL in Israel. Firstly Bartal *et al* (1978) found 80% of 288 cases of CLL in Israelis were in Ashkenazi Jews (originated from European countries) and 15% were in Sephardic Jews (from Asia and Africa). Shvidel *et al* (1998)

have since reported 302 cases of CLL and related disorders and found that 67% were Ashkenazi Jews and 31% were Sephardic Jews. There may be many reasons for this shift including increased access to health care and an increase in routine blood counts.

There is a higher incidence of myeloma in black-Americans than white-Americans (Walker *et al*, 1995). Landgren *et al* (2006) looked at the prevalence of monoclonal gammopathy of undetermined significance (MGUS) among four million African-American and white male veterans admitted to Veterans Affairs hospitals and found the age-adjusted prevalence ratio of MGUS was 3.0 (2.7-3.3 95% confidence interval) in African-Americans compared to whites. They found similar rates of progression to myeloma in the two groups of MGUS patients and therefore concluded that the increased incidence of myeloma amongst black Africans is related to an increased incidence of MGUS and not increased transformation. It has been suggested that racial differences in immunogenetic factors may affect the risk of developing both MGUS and myeloma (Morgan *et al*, 2002). It has also been hypothesised that the increased rate of myeloma in African-Americans might be related to sickling traits with interleukin-6 as a common link (Adegoke, 2003).

Au *et al* (2004, 2005) report on the incidence of HL and NHL in Chinese migrants to British Columbia. 24 cases of HL were identified in Chinese migrants between 1970-1994 with a crude and age-adjusted incidence rate of 0.91 and 1.14 per 100 000 per year (Au *et al*, 2004). During the same period the provincial crude and age-adjusted incidence rates were 5.2 and 4.87 per 100 000 per year and the figures for the Hong Kong Chinese population were 0.32 and 0.31 per 100 000 per year. They conclude that although the numbers are small and there may be many confounding factors there appears to be both genetic and environmental factors involved as the incidence remains lower in the migrants than the provincial population, but is higher than that for a similar ethnic population that remained in the East.

211 cases of NHL were identified in Chinese migrants to British Columbia between 1980 and 1997 (Au *et al*, 2005). The age-standardised incidence rate was 7.11 per 100 000 per

year compared with Hong Kong and British Columbian rates of 7.91 and 11.88 respectively. They found that the standardized rates of FL remained low, but that the incidence of gastric and nasal NK/T-cell lymphoma was lower than expected amongst the migrants. They conclude that genetic factors appear to be stronger than environmental factors in governing the overall incidence of NHL in Chinese, but that environmental factors may have played a role in the reduced rates of some lymphoma subtypes.

Mills *et al* (2005) looked at the incidence of cancer amongst the Hmong immigrant group, from Southeast Asia, living in California. They found that the age-adjusted cancer rate for the Hmong was 284 per 100 000 population compared to 362.6 and 478 per 100 000 population for the Asian/Pacific Islander and non-Hispanic white populations also living in California, respectively. The age-adjusted incidence rates of cancer in the Hmong were increased for hepatic, gastric, cervical and nasopharyngeal cancers, leukaemia and NHL. Rates were lower among the Hmong for colorectal, lung, breast and prostate cancers.

Lanier *et al* (2003) looked at rates of childhood cancer amongst Alaskan natives and found that once hepatocellular carcinoma secondary to hepatitis B infection was excluded the overall rate of cancer in these children was less than that in white American children and that the rate of HL was particularly low. This was reassuring especially given the concerns regarding ionizing radiation in the area.

People of Native American descent in Central and South America are genetically linked to Asians and also have an increased risk for NK/T-cell neoplasms, however the evidence points towards EBV being the important cofactor as opposed to race (Arber *et al*, 1993; Elenitoba-Johnson *et al*, 1998).

Links between LPDs and sex

NHL generally has a slight male predominance (52-55% male); the main exceptions are mediastinal large B-cell lymphoma which has a moderate female predominance (66%), FL which has a mild female predominance (58%) and MCL where there is a striking male predominance (74%) (Jaffe *et al*, 2001). In ALL there is a male predominance except in

infancy, where there is a slight female predominance (Biondi *et al*, 2000). A male predominance occurs at all ages in HL, but is most pronounced in childhood cases (Lichtman *et al*, 2006).

Links between LPDs and socioeconomic factors

Links between socioeconomic factors and the development of LPDs have been proposed, in particular for HL (Cartwright & Watkins, 2004). A link between HL and socioeconomic status has been postulated since higher intelligence quotients were noted in World War II military personnel with HL compared to the general army population (LeShan *et al*, 1959). A study looking at the incidence rates of HL in England and Wales found that proximity to built-up areas and higher socio-economic status were significant risk factors for the development of HL (Alexander *et al*, 1991). The link also appears to vary between sub-types and ages groups of patients with HL. Clarke *et al* (2005) found that the link between HL and socioeconomic status in California was strongest for young adults with NS HL and older white and Hispanic male patients with MC HL. They propose that etiologically relevant exposures for young adult HL patients may be linked to race or ethnicity as opposed to socioeconomic status per se.

A link between reduced exposure to infection in early childhood, secondary to improved socioeconomic status, and the increasing incidence of both NHL and paediatric ALL in developed countries has been postulated (Vineis *et al*, 2000a; Smith *et al*, 1998). Socioeconomic status is also linked to H. pylori prevalence with potential implications for the development of gastric MALT-lymphoma and SMZL (Goodman & Correa, 2000; De Sanjose *et al*, 2004). Socioeconomic status does not appear to influence delayed diagnosis of NHL in England – the only significant factor resulting in delayed diagnosis was younger age (Neal & Allgar, 2005).

Links between LPDs and other factors

Autoimmune disorders are associated with a slightly increased risk of NHL; this risk is higher in patients with primary Sjogren syndrome than those with rheumatoid arthritis or systemic lupus erythematosus (SLE) (Zintzaras et al, 2005). The link between coeliac disease and enteropathy-type T-cell lymphoma is well documented, but Smedby *et al*

(2005a) reviewed 11 650 patients with coeliac disease and found they also had an increased incidence of B-cell NHL.

In a Swedish population-based case-control study a twofold increased risk of NHL and HL was found for individuals with a family history of haematopoietic malignancy, with the link being stronger between siblings than parent-child (Chang *et al*, 2005b). Familial CLL is well documented and at a molecular level is indistinguishable from sporadic CLL favouring a genetic, rather than an environmental, aetiology (Crowther-Swanepoel *et al*, 2008).

Links between LPDs and many other factors have been studied. Some of these are summarized in table 1.6.

Table 1.6 – Factors linked or not linked to the development of LPDs

References	**Factors felt to be linked to the development of LPDs**	**Factors felt unlikely to be linked to the development of LPDs**
Vineis *et al,* 2000b	Previous polio, SLE, hepatitis, malaria in childhood; history of parental tuberculosis	Diabetes, tonsillectomy or adenoidectomy
Cerhan *et al*, 1997, 2001, 2002, 2003	Adult-onset diabetes, previous cancer and previous blood transfusion, current hormone replacement therpay, Non-steroidal anti-inflammatory drugs (NSAIDs)	Menstrual and reproductive factors, previous HRT
Tavani *et al*, 1999; Chow & Holly, 2002a, 2002b; Zhu *et al*, 2003		Blood transfusion

Table 1.6 – Factors linked or not linked to the development of LPDs continued

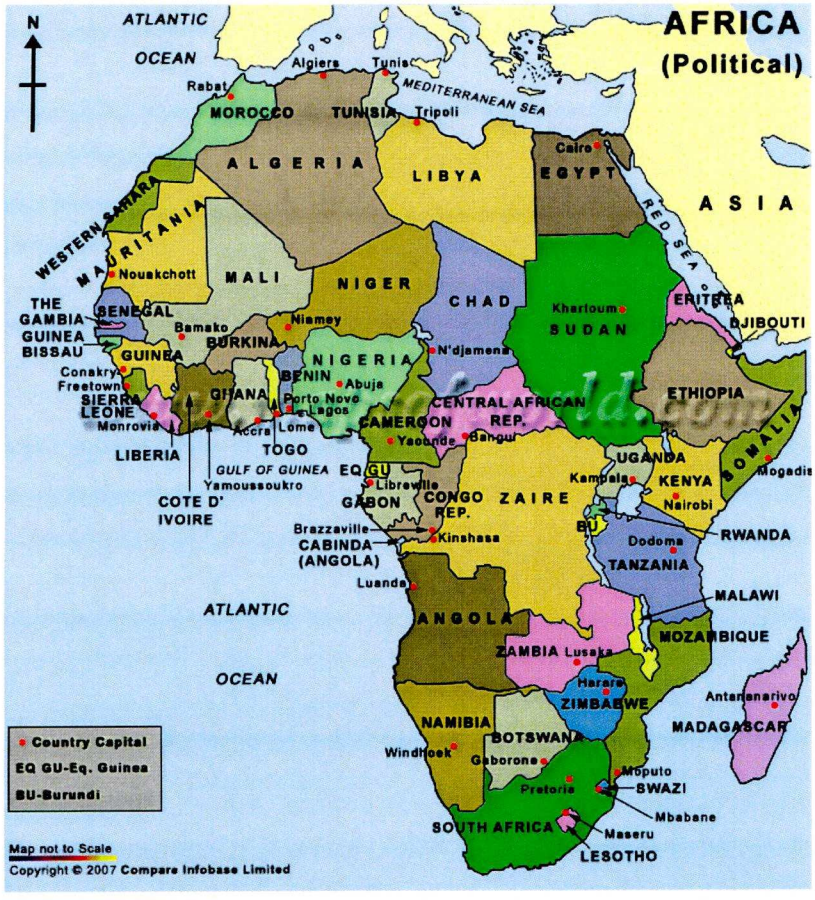
References	Factors felt to be linked to the development of LPDs	Factors felt unlikely to be linked to the development of LPDs
Chang *et al* 2005a	>10 courses of antibiotics in adulthood, high cumulative use of NSAIDs	Other drugs
Chang *et al*, 2005c; Chiu *et al*, 1996; Tavani *et al*, 1997; Zhang *et al*, 1999, 2000	Diet containing a lot of dairy products and fried red meat	Women whose diet contained a lot of fruit and vegetables
Zheng *et al*, 1996		Tea consumption
Morton *et al*, 2005; Briggs *et al*, 2002a		Alcohol ?wine more protective than beer
Briggs, 2002b; Peach & Barnett, 2001	Smoking with HL	Smoking with NHL
Chang *et al*, 2005d; Cerhan *et al*, 2002; Kasim *et al*, 2004	Raised body mass index with CLL	Raised body mass index with lymphoma
Fritschi *et al,* 2005	Solvents, wood dust	Other organic dusts, metals and polychlorinated biphenyls
Wong & Raabe, 2000		Benzene exposure
De Roos *et al*, 2003	Pesticides	
Baris *et al*, 2004	Farmers, pharmacists and roofers	
Freedman *et al*, 1997; Adami *et al*, 1999; van Wijngaarden *et al*, 2001; Hughes *et al*, 2004; Smedby *et al*, 2005b		Sunlight
van den Bosch, 2004	Euphorbia tirucalli – BL	

LPDs in developing countries

In May 2003 the International Network of Cancer Treatment and Research held a workshop looking at lymphoma diagnoses from several developing countries (India, Egypt, Kuwait, Pakistan and Turkey) (Naresh *et al*, 2004). The aim was to highlight variations in the spectrum of lymphomas and to identify areas for research. Most of the centres involved in this conference had used some immunohistochemistry, but no molecular data was available. 82-88% of the NHLs were B-cell in origin. There was little variation between countries regarding the proportions of CLL, mantle cell lymphoma and plasmacytoma cases, but there was a significant variation between countries regarding the proportion of cases of follicular lymphoma. There were relatively high frequencies of BL in Egypt and of DLBL and precursor T lymphoblastic lymphoma in India. It was concluded that since there were differences in proportions of lymphoma cases amongst different developing countries, and further differences between developing countries and that found in the rest of the world, further research looking at lymphomas in developing countries should provide valuable insights into lymphomagenesis.

There is very little prospective published data on adult LPDs from sub-Saharan Africa other than data from South Africa. South Africa has a more advanced healthcare system than the rest of sub-Saharan Africa and malaria is not endemic so this data is not readily translatable to Central, East and West Africa. The following subsections therefore present the published data on LPDs diagnosed in sub-Saharan Africa other than South Africa. Where possible, paediatric cases have been excluded so that the data can be compared to the project data. Figure 1.3 shows a map of Africa.

Figure 1.3 – Map of Africa (www.mapsofworld.com)



Current knowledge about lymphomas in sub-Saharan Africa

Kenya

A retrospective study of 207 cases of NHL, aged over 13 years, presenting in Nairobi, Kenya between 1990 and 2000 found that 41% were not properly classified histologically and only 15.3% of 105 evaluable patients were followed up for at least 36 months as most were lost to follow-up (Othieno-Abinya *et al*, 2004). They also found that very few patients could afford standard treatment, but that over the ten year period the treatment and prognosis of patients presenting with NHL improved. The same authors also reviewed 111 cases of HL presenting during the same period of time and found that 14.2% were lymphocyte predominate (LP), 23.6% were NS, 26.4% were MC, 17% were lymphocyte depleted (LD) and 18.8% were histologically unclassifiable (Othieno-Abinya *et al*, 2005). 24.5% of cases were stage IIIB or IV; 1st line treatment was evaluable in 99 patients of which 87 had chemotherapy alone, 9 had radiotherapy alone, and 1 had both chemotherapy and radiotherapy; median duration of follow-up was 10 months (range 0.5-122 months). From the same centre, Mwanda *et al* (2004) report 54 cases of HIV-associated NHL presenting between 2001 and 2003. 15 were BL, 35 were large cell lymphoma and 4 were intermediate grade lymphoma; primary sites at presentation were: 16 (30%) peripheral nodes, 15 (28%) abdominal, 11 (20%) pectoral/chest wall, 8 (15%) CNS, 4 (7%) systemic.

The lymphoma diagnoses on 73 paraffin blocks from biopsies taken in 40 Kenyan mission hospitals between March 1993 and November 1994, were reviewed, immunophenotyped and studied for EBV in the United States (Cool & Bitter, 1997). The male:female (M:F) ratio was 2.5:1 and the median age was 35 years (range 4-97 years, 19 patients <16 years) - age was not available in 9 patients. There were 21 cases of BL (11 paediatric, 5 adult and 5 age unknown) and 13 cases of HL (6 NS, 4 MC, 1 LD, 1 LP) with an apparently preserved bi-modal age distribution. The other diagnoses were 11 DLBL, 9 small lymphocytic lymphoma, 6 Burkitt's-like high grade B-cell lymphoma, 2 FL, 2 MCL, 1 MZL, 6 precursor T-cell lymphoblastic lymphoma/leuakemia and 2 PTCL. Of 39 cases that were assessable for EBV, 22 were positive, including all 17 assessable cases of BL.

Uganda

A review of the biopsies of cases of lymphoma diagnosed in Uganda between 1966 and 1973 showed that there was a low frequency of follicle-centre-cell lymphomas and that BL as well as non-Burkitt high grade NHL had a similar distribution to malaria (Schmauz *et al*, 1990).

Blocks from 600 cases of NHL from Uganda that had been diagnosed between 1991 and 2000 were reviewed, of these 129 blocks were felt suitable for immunohistochemistry, of these 95 (74%) were BL, 19 (15%) were DLBL, 4 (3%) were MCL and 1 (0.8%) was B-cell lymphoblastic lymphoma (Tumwine *et al*, 2008). 200 cases of HL that had been diagnosed between 1980 and 2000 were also reviewed first with repeat histology and then with immunohistochemistry (CD45, CD15, CD30 and CD20) (Tumwine, 2004). On histology it was possible to confirm and classify the diagnosis of HL in 131 cases, 29 cases were unsuitable for assessment and immunohistochemistry was needed to make the full diagnosis on 40 cases. The sensitivity and specificity of routine histology using haematoxylin and eosin (H&E) stain was 76.6% and 92.3% respectively (Tumwine *et al*, 2003).

Ethiopia

83 lymphomas diagnosed in Western Ethiopia between 1988 and 1999 were reviewed – 21 (25.3%) were HL, 61 (73.5%) were NHL and 1 case was unspecified; the M:F ratio was 3.4:1 (Getachew, 2001). Of the HL 38.1% were LP, 33.3% were MC, 14.2% were LD, 4.8% were NS and 9.5% were unclassified; of the NHL 6.6% were BL, 34.4% were high grade NHL, 9.8% were intermediate grade NHL, 32.8% were low grade NHL, 6.6% were mycosis fungoides and 9.8% were unclassifiable.

Sudan

A prospective study of 92 patients presenting with cervical lymphadenopathy in Sudan showed that 45% were due to tuberculosis, 16% were lymphoma, 18% were a secondary malignancy (Kheiry & Ahmed, 1992). It was not possible to delineate between the patients with tuberculosis and lymphoma on symptoms alone emphasizing the need for histological diagnosis prior to anti-tuberculous therapy.

Rwanda

115 lymphoma biopsies from Rwanda between 1979 and 1987 were also reviewed - 83.5% were NHL, of which 94.8% were B-cell in origin, and 16.5% were HL, of which 57.9% were MC and 83.3% in the 15-40 year age group (Ngendahayo & Schmauz, 1992). Of the B-cell NHLs 47.3% were extranodal and 48.4% were high grade with an increase in high grade and in particular BL cases in the second half of the study period.

Tanzania

Kaaya *et al* (2006) prospectively investigated 22 Tanzanian patients with lymphoma (HIV serology, immunohistochemistry, EBV/HHV8/HHV6/cytomegalovirus by PCR and EBV by in situ hybridisation). Of the 22 patients, 10 were >12 years old, of which 6 had DLBL (2/5 HIV positive, 2/4 EBV positive), 3 had LD HL (2/3 HIV positive, 3/3 EBV positive), 2 had MC HL (0/2 HIV positive, 1/1 EBV positive) and 1 had precursor B-lymphoblastic lymphoma. 10/22 (45%) lymphomas were positive for HHV8 by PCR, but not by immunostaining for HHV8 LANA and PCR positivity was not specific for a particular type of lymphoma nor for HIV status so they conclude that HHV8 was not associated with HIV-associated lymphoma pathogenesis. They also retrospectively looked at registry data for 167 lymphomas diagnosed 1989-1996 and found only a slight increase in incidence of lymphoma during the 7 year period, despite the HIV epidemic.

Zimbabwe

Levy prospectively investigated LPDs in Zimbabwe for 3 years and found less BL than that reported from African countries nearer the equator, very little FL, but a preservation of the bimodal age distribution of HL (Levy, 1988 a, b, c).

Nigeria

A review of 129 lymph node biopsies in Jos, Nigeria found that, once sarcomas and carcinomas were excluded, tuberculosis accounted for 48% of the cases, NHL 22.7%, HL 6% and non-diagnostic 20.8% (Obafunwa *et al*, 1992). Another Nigerian review of 143 cases of lymphoma in adult patients presenting to the teaching hospital in Ife-Ife between 1989 and 1998 found that 74% of cases were NHL and 26% HL with intermediate grade tumours, especially DLBL, predominating amongst the cases of NHL and MC amongst

the cases of HL (Adelusola *et al*, 2001). The same author reviewed cases of non-Burkitt's NHL presenting to the same institution over a 5 year period and found that of 53 cases 54.7% were intermediate grade, 24.5% low grade and 20.8% high grade (Adelusola & Durosinmi, 2004).

Omoti & Halim (2005) reviewed 205 cases, over the age of 18 years, with a histological diagnosis of lymphoma in Benin City, Nigeria between 1990 and 2003. Classified according to the Working Formulation 20 were low grade NHL, 70 were intermediate grade NHL, 50 were high grade NHL, 20 were unclassifiable NHL, 10 were other NHLs and 35 were HL. The M:F ratio was 1:1.9, the median age for NHL was 39 years and for HL was 33 years. Of the patients with NHL 82.4% were stage 3-4 and 79.4% were in the intermediate-high risk group (3-5) by international prognostic index (IPI).

Retrospective immunophenotyping (CD20, CD3, CD68) of 100 cases of NHL that had been fixed and processed in Ibadan, Nigeria showed a clonal population in 87 - 75 B-cell (CD 20 positive, CD3 negative), 12 T-cell (CD3 positive, CD20 negative) (Thomas *et al*, 1991). In 4 cases of high grade lymphoma the tumour cells did not stain although background reactive cells stained and immunostaining failed completely in 9 cases. Of the B-cell NHLs most were high grade and there were very few cases of FL (Thomas, 1992).

Mali

14 cases of CTCL presenting between 1992 and 1994 were reviewed in Bamako, Mali – there were 3 cases of ATLL associated with HTLV-1, 3 of Sezary syndrome, 2 of mycosis fungoides, 5 of pleomorphic cutaneous lymphoma and a final case where it was not possible to differentiate between mycosis fungoides and pleomorphic cutaneous lymphoma (Fouchard *et al*, 1998).

Current knowledge about lymphomas in Ghana

Regarding lymphoma data from Ghana a review of 330 autopsies performed during an 11 month period at KBTH in the 1960s found that there were 23 cases of malignant

neoplasm, 7 of which were lymphomas (Younn, 1964). The median age of the patients was 28 years (range 11-43 years), the retroperitoneum and stomach were commonly involved and similarities were noted between these tumours and 'jaw tumours of Burkitt'.

Two papers, published a decade later, reviewed surgical material that had been sent to KBTH between 1966 and 1971 (Anim, 1973a, 1973b). 391 cases were seen – 134 BL, 70 HL, 68 lymphosarcoma, 110 reticulum cell sarcoma and 9 unclassified (Anim, 1973a). BL occurred predominately 1-20 year age group with equal sex distribution whereas the M:F ratio for both HL and lymphosarcoma was 3:1 and for reticulum cell sarcoma it was 2:1. Most cases were under 50 years of age with only 8 cases over the age of 70. There was an even distribution amongst the 4 main tribal groups when compared to the 1960 census. The second paper focuses on HL with a review of 45 of the 70 cases of which 35 were MC, 6 were NS, 3 were lymphocyte predominate and 1 was LD (Anim, 1973b).

Wiredu and Armah (2006) reviewed 3659 cancer deaths at KBTH between 1991 and 2000 from autopsy records and registered causes of death. The M:F ratio was 1.2:1 with a median age in men of 50 years and 48years in women. Haematological malignancy was the second commonest malignant cause of death in women (14.7%) second to breast cancer (17.2%) and third commonest in men (15.6%) second to liver (21.2%) and prostate (17.4%) cancers. The frequencies of the different haematological malignancies are not mentioned however.

Current knowledge about lymphoid leukaemias in sub-Saharan Africa

60 of the 167 (36.4%) cases of leukaemia diagnosed between 1995 and 2005 in Benin City, Nigeria, were CLL (morphological diagnosis as immunophenotyping was not available locally) (Omoti *et al* 2007). The M:F ratio of the CLL patients was 1:3 with a median age of 56 years (range 20-84 years); interestingly the male patients (mean age 63.4 years) were generally older than the female patients (mean age 53 years). 91.7% had lymphadenopathy, 58.3% anaemia, 58.3% abdominal swelling, 50% splenomegaly and only 7 patients (11.7%) were Binet stage A; the 2 year survival rate (from date of diagnosis to date of death/last documented visit) was only 28%.

A review of 102 cases of CLL presenting to the Tikur Anbessa hospital, Addis Ababa, Ethiopia found the male to female ratio to be 3.6:1 and the age range 35-91 years with 56% of cases presenting with Rai stage III-IV disease (Shamebo & Gebremedhin, 1996). Presenting symptoms were similar to those seen elsewhere, but they again found a high default rate (69/102).

Of the 95 cases of leukaemia diagnosed between 1994 and 1998 in Blantyre, Malawi there were 14 cases of ALL, 22 of CLL and 2 cases of hairy cell leukaemia (HCL) with most of the cases of ALL occurring in children (Mukiibi *et al*, 2001). Macharia (1996) reviewed the available data looking at prognostic factors in childhood ALL presenting in Africa compared to the known data for developed countries and found that there was a higher white cell count (WCC) at presentation, a greater proportion with T-cell immunophenotype (60% versus 17%) and CNS and mediastinal involvement.

Current knowledge about lymphoid leukaemias in Ghana

20 cases of paediatric ALL presenting to KBTH were matched by age and sex to cases presenting to Hamburg Children's Hospital, Germany (Ekem, 2000). The WCC at presentation was not significantly different between the 2 groups. The Ghanaian children presented with more organomegaly, were treated less intensively, had a greater default rate (60%) and a shorter duration of follow-up (6 months versus 69 months) than the German children. 75% of the Ghanaian children and all the German children went into remission.

Current knowledge about myeloma in sub-Saharan Africa

A review of 27 patients with myeloma presenting in Ife-Ife, Nigeria found similar presenting features and age/sex distribution to that found in developed countries, but with relatively short follow-up due to a high default rate as well as mortality due to renal failure and anaemia (Salawu & Durosinni, 2005). Omoti & Omuemu (2007) presented 30 patients diagnosed with myeloma in Benin City, Nigeria between 1993 and 2003. The M:F ratio was 2:1, the median age was 54 years and the median survival was 3 months

with only 4 patients (13.3%) alive at 2 years. The authors felt that late presentation and poor compliance with treatment contributed to the poor survival of patients. Similar demographics and problems relating to late presentation were reported in 22 myeloma patients presenting over a 10 year period in Dakar (Pouye *et al*, 2004).

Current knowledge about myeloma in Ghana

There is no published data regarding myeloma in Ghana, however Landgren *et al* (2007) found that 59% of 917 Ghanaian men, aged 50-70 years, in Accra had a monoclonal band on serum electropheresis giving an age-adjusted prevalence of 5.84 per 100 persons which is 1.97 times higher than that found in a similar study of men from Minnesota analysed in the same laboratory (Kyle *et al*, 2006). None of the Ghanaian patients had evidence of myeloma from their clinical history although further tests were not performed.

Factors affecting the diagnosis and epidemiology of LPDs in developing countries

Infection

People living in developing countries are exposed to more frequent and a greater range of infections than those living in developed countries. For example, in developed countries 10-20% of adults are infected with H. pylori by the age of 30, whereas in developing countries most individuals are infected by the time they reach adolescence (Pounder & Ng, 1995; Goodman & Correa, 2000). Some infections that have been virtually eradicated from most developed countries due to vaccination and vector control programmes, such as polio and malaria, continue to be a problem in developing countries. People living in developing countries also have less access to health care and thus infections may not be as promptly or thoroughly treated. Given the strong links between various infections and LPDs this is likely to significantly impact on the epidemiology of LPDs in developing countries.

>90% of the world's adult population are EBV seropositive; primary infection in developed countries usually occurs in adolescence whereas in developing countries primary infection tends to occur in childhood (de-Thé, 1976; Takenuchi *et al*, 2006).

Rasti *et al* (2005) looked at cell-free EBV-DNA in plasma from 73 Ghanaian children (32 with acute malaria, 41 age-matched without malaria), as well as adult Ghanaians and Italian children as controls, and found viral DNA in 47% of the Ghanaian children with malaria and in 34% of the Ghanaian children without malaria, but not in the plasma of the Ghanaian adults or Italian children. This is further evidence that the epidemiology of EBV infection and persistence varies in different areas of the world; viral reactivation in children living in malaria-endemic areas may contribute to the increased risk of endemic BL.

The increased incidence of HIV in many developing countries is having an impact on the development of cancers, especially LPDs (Mbanya, 2002; Mbulaiteye *et al*, 2005; Orem *et al*, 2006). Whereas in many developed countries the availability of HAART and increasing experience treating HIV related LPDs is improving outcome, in developing countries these drugs are not yet as widely available nor are treatment options for such aggressive lymphomas although feasibility studies of simplified chemotherapy regimens are underway (Lim & Levine, 2005; Kaaya *et al*, 2006; Orem *et al*, 2005 and 2006).

The Demographic and Health surveys assessed HIV prevalence in Ghana in 2003. 85% of 5345 men (aged 15-59 years) and 5949 women (aged 15-49 years) eligible for testing were tested; the adjusted prevalence was 1.69 in men and 2.71 in women (the 95% confidence intervals were 1.38-2.00 and 2.32-3.10 respectively) (Mishra *et al*, 2006). This is slightly lower than the 3.2-3.6% HIV prevalence rates usually quoted for Ghana which may reflect the fact that most HIV prevalence rates are derived from surveillance systems in antenatal clinics which are useful for tracking trends in infection, but are a less accurate reflection of the population as a whole (2005 Programme of Work, www.ghanahealthservice.org; Mishra *et al*, 2006; Aikins, 2007).

A study of prison inmates and officers in Ghana with an uptake of 18% (1336) of 7652 eligible inmates and 21% (445) of 2139 eligible officers showed an HIV seroprevalence of 5.9% and 4.9% respectively, syphilis seroprevalence of 16.5% and 7.9% respectively and HCV seroprevalence of 18.7% in both groups (Adjei *et al*, 2007; Adjei *et al*, 2008).

As mentioned previously HTLV-1 is strongly associated with the development of ATLL. A study of 3763 Ghanaians showed that the prevalence of HTLV-1 antibodies was 1-2% with no geographical variation; this prevalence was no different amongst 124 patients with haematological malignancies including lymphoma (Biggar *et al*, 1993).

As also mentioned previously HHV8 is associated with some HIV-associated lymphomas as well as some occurring in the absence of HIV infection. A study looking at seroprevalence to HHV8 in various countries showed that Ghana had the highest rate of positivity at 42% compared with 1.3% in Trinidad, 4% in Thailand, India and Malaysia and 5.2% in the United States (Ablasi *et al*, 1999).

Socioeconomic factors, supportive care and intensive therapies

Socioeconomic factors also play a major role in the diagnosis and treatment of LPDs in developing countries (Eden, 2002; Metzger *et al*, 2003; Hesseling *et al*, 2003; Meremikwu *et al*, 2005; Howard *et al*, 2005). The difference is particularly striking in diseases where, with modern diagnostic techniques and therapy, excellent results are often achieved with good long term prognosis – for example paediatric ALL and endemic BL.

Hesseling *et al* (2003) report using a modified version of the LMB 89 group B protocol for the treatment of endemic paediatric BL in Malawi at a cost of <$1000 per child. The original LMB 89 group B protocol has been shown to cure >80% of children with stage III BL elsewhere, but involves more intensive treatment and requires greater levels of supportive care than that available in Malawi. 44 children (10 stage I, 5 stage II and 29 stage III), with a median age of 7.2 years, were treated with an overall projected Kaplan-Meier event free survival (EFS) of 57% at 1 year (90% for stage I, 52% for stage III). There were 10 deaths due to toxicity and delays in supportive care, 6 due to relapse and 2 due to progressive disease highlighting the need for adequate supportive care in such a setting.

Meremikwu *et al* (2005) report a similar cohort of 41 children with suspected endemic BL in Nigeria. They highlight other problems related to socioeconomic status, in particular presentation with advanced stage disease (20/41) and lack of money to pay for basic diagnostic tests (9/41), for chemotherapy (8/41 – 1 patient was treated empirically based on a clinical diagnosis) and for ongoing care once chemotherapy started (13/33).

Metzger *et al* (2003) looked at the outcome of 168 children with ALL treated in Honduras and found that abandonment of treatment (38/168) was the main cause of treatment failure and was associated with prolonged travel time to the treatment centre and child age <4.5 years, with a further 35/168 patients dying from treatment-related effects.

Howard *et al* (2005) discuss the strategies needed to improve outcomes of children with cancer in low-income countries – the development of paediatric cancer units, for example, is crucial in order to educate staff and ensure uniform levels of care, use of minimally toxic regimens, parent education and improved supportive care and provision of housing and economic support to minimise abandonment of therapy.

In most developing countries patients, or their families, have to pay for all their diagnostic tests; therefore it is often difficult to diagnose or stage patients even when diagnostic facilities are available in that country. This then makes treatment decisions even more difficult. Earlier in the chapter the difficulty making a complete histological diagnosis based on histology alone was shown with many studies finding 10-20% of lymphomas unclassifiable on histology (Kheiry & Ahmed, 1992; Getachew, 2001; Tumwine *et al*, 2003; Othieno-Abinya *et al*, 2004; Tumwine, 2004; Omoti & Halim, 2005).

Biopsy and histology, although cheaper than immunohistochemistry, are too expensive for many patients in developing countries and FNA is often used. A prospective study in Lusaka, Zambia used a modified FNA technique using a 19 gauge needle to assess lymph node aspirates; this allowed more sample to be aspirated for review by the hospital

histopathologist as they did not have a cytologist (Patil & Bem, 1993). Aspirates were performed by the surgeon immediately prior to surgical biopsy of the node; the slides were stained with H&E and Ziehl Neelson stains and the results compared with the histological diagnosis. The positive predictive values were 96.8% for tuberculous lymphadenitis, 83.8% for primary HIV lymphadenopathy, 50% for Kaposi's disease and 71.4% for malignancy. They conclude that wide needle aspiration was a useful investigation that would have avoided the need for a biopsy in large proportion of patients even in the absence of a qualified cytologist. Where chemotherapy and radiotherapy are available however, it is important to have a more precise diagnosis than simply malignancy and so a lymph node biopsy is essential in these patients.

A review of the results of 808 lymph node FNAs at Jimma Teaching Hospital, Ethiopia found the following: 536 (66.3%) tuberculous lymphadenitis, 155 (19.2%) reactive, 35 (4.3%) granulomatous inflammation, 27 (3.3%) pyogenic lymphadenitis, 22 (2.7%) NHL, 15 (1.9%) HL, 18 (2.2%) metastatic tumours (Bezabih & Mariam, 2003). The following diagnostic criteria were used: NHL - cells were largely monotonous non-cohesive neoplastic lymphoid cells; HL - Reed-Sternberg cells with the characteristic background cells; metastatic carcinoma - loose to tight aggregates of epithelial cells. Again, when considering chemotherapy and radiotherapy a histological diagnosis is crucial.

Diagnosis of indolent LPDs

In developed countries there has been a large increase in the number of laboratory, radiology and endoscopic investigations performed per capita. In developing countries the number of tests performed has also increased, but routine testing is not done. Asymptomatic disease such as asymptomatic CLL, smouldering myeloma and other asymptomatic low grade LPDs, as well as pre-neoplastic disorders such as MGUS, are therefore rarely diagnosed in developing countries.

Other Factors

Factors that were previously confined to developed countries, such as obesity, smoking, alcohol and pollution/exposure to toxic substances, are now causing serious public health problems in developing countries (Sobngwi *et al*, 2004; Boutayeb, 2006). Many factors

are responsible for these changes including migration to urban areas, industrialisation, shifts in diet away from locally produced food to imported food stuffs and a lack of control regarding the use of chemicals. El-Sadek & Hassan (1999) looked at Egyptian farm workers exposed to pesticides compared to controls and found that although the farm workers had higher lymphocyte counts than the non farm workers, and also higher white cell and platelet counts, they did not have a statistically significantly increased risk of CLL.

Previous work on Tropical Splenic Lymphoma and Hyper-reactive Malarial Splenomegaly

Massive splenomegaly, splenomegaly palpable at least ten centimetres below the costal margin, is a common finding in tropical countries. The differential diagnosis for these patients includes HMS, schistosomiasis, visceral leishmaniasis, haemoglobinopathies including thalassaemia major, chronic myeloid leukaemia and myelofibrosis. Other possible diagnoses are splenic cysts, splenic tumours and lipid storage diseases. Causes of moderate splenomegaly (palpable less than ten centimetres below the left costal margin) include chronic haemolysis (common causes of this include recurrent malaria and haemoglobinopathies), portal hypertension, leukaemias, lymphomas and polycythaemia vera (Fleming, 1996).

These conditions can be difficult to investigate in a tropical setting due to lack of diagnostic techniques. Clinicians use their knowledge of local infections supplemented, where available, by white cell counts and blood film findings. Undiagnosed cases were previously grouped together as 'idiopathic tropical splenomegaly' – from this group HMS and TSL have emerged.

HMS was previously known as tropical splenomegaly syndrome, but was renamed in 1983 as the links with malaria in its pathogenesis became clear (Bryceson *et al*, 1983). Criteria for the diagnosis of HMS were first published in 1979, revised in 1981 and reviewed in 1997 (Greenwood & Fakunle, 1979; Fakunle, 1981; Bates & Bedu-Addo, 1997b). Criteria for a diagnosis of HMS include: splenomegaly of at least ten centimetres

below the costal margin, a serum IgM concentration at least 2SD above the normal mean concentration for the area, a sustained response to malaria prophylaxis with a reduction in the size of the spleen of at least 40%, evidence of the polyclonal nature of the lymphocytes (Bates & Bedu-Addo, 1997b).

Polyclonal rearrangements of the immunoglobulin gene have been initially demonstrated using Southern blotting and subsequently with PCR (Bates *et al*, 1991; Jimmy *et al*, 1996). Due to a lack of diagnostic facilities, however, it has often been difficult to delineate HMS patients from those with LPDs. Over an eleven year period (1986-1997) 395 patients were referred to the 'big spleen' clinic at KATH (Bedu-Addo & Bates, 2002). 221 (56%) of these patients had adequate laboratory data and follow-up to allow the authors to make a meaningful assessment. Of these 91 patients (41%) were felt to have HMS, 48 (22%) to have B-LPDs, 32 (14%) to have other haematological disorders and in 50 patients (23%) no diagnosis was made. The diagnosis of HMS was made by excluding other disorders both clinically and by showing no evidence of a clonal population of lymphocytes (by Southern blotting, PCR or immunophenotyping) followed by demonstration of a sustained reduction in spleen size by 40% when treated with anti-malarial drugs. This study demonstrates some of the difficulties inherent in diagnosing HMS – namely the lack of a single, locally available diagnostic test and difficulties regarding follow-up of patients in a tropical setting.

There has previously been some confusion regarding African patients that were felt to have CLL. It was noticed that there appeared to be two groups. The first group were mostly elderly males, with a marked lymphocytosis and some lymphadenopathy and the second group were younger, predominately female and had splenomegaly (Essien, 1976; Fleming, 1985). A link between the second group of 'CLL' patients and patients with HMS was first noticed in 1992 with the 'CLL' patients being felt to have a type of splenic lymphoma with villous lymphocytes (SLVL) (Bates *et al*, 1992). Further investigation has shown marked similarities between Ghanaian patients with HMS and 'SLVL'. Firstly both groups of patients have villous lymphocytes on their PB films and are predominately female (Bates & Bedu-Addo, 1997a). Secondly both groups have markedly raised total

IgM and antimalarial antibodies (Wallace *et al,* 1998). Finally both groups had markedly raised EBV antibodies without an associated increase of viral genomes in the blood, but no increase in HCV antibodies or HHV8 antibodies (Bates *et al*, 2001).

Further work investigating the Ghanaian patients with 'SLVL' has shown that the cell of origin appears to be a naïve B-cell as opposed to the post-germinal centre B-cell that is usually the cell of origin in patients with SLVL (Zhu *et al*, 1999). As a result the Ghanaian 'SLVLs' have been named TSL pending further studies.

Due to the similarities between patients with HMS and those with TSL, and with the improved knowledge regarding mechanisms of lymphomagenesis, it has been postulated that HMS can evolve to become TSL (Bates& Bedu-Addo, 1997b). Further work to describe TSL in greater detail and diagnose patients with certainty on a routine basis had not been possible due to a lack of diagnostic facilities.

Models that exist for collaborative diagnosis between developed and developing countries

Collaborations between developing and developed countries in diagnosing and treating paediatric acute lymphoblastic leukaemia

Such collaborations can focus on training staff and provision of treatment and supportive care such as that reported firstly by Magrath between three Indian centres (Cancer Institute Madras, Tata Memorial Hospital Bombay and the All India Institute of Medical Sciences New Delhi) and the National Cancer Institute, Bethesda, USA, and secondly by Masera, between La Mascota paediatric hospital in Managua, Nicaragua, and hospitals in Monza and Milan, Italy and Bellinzona, Switzerland (Magrath *et al*, 2005; Masera *et al*, 1998). These collaborations focused on paediatric ALL which is a significant cause of paediatric mortality in developed countries and is emerging as a significant cause in developing countries as preventable causes of paediatric mortality diminish, such as infection and malnutrition. In these collaborative examples diagnosis was made locally although support in terms of training was provided.

Bonilla and Ribeiro report a similar collaboration between Benjamin Bloom Hospital, El Salvador and St Jude Children's Research Hospital (SJCRH), Memphis, USA (Greenberg *et al*, 2001; Bonilla *et al*, 2000; Ribeiro *et al*, 1996). Primary objectives of this study were to describe the clinical and biologic features of childhood ALL in El Salvador and to report the results of efforts aimed at developing effective treatment regimens for ALL in El Salvador through a partnership with SJCRH (Bonilla *et al* 2000). The feasibility of performing special laboratory techniques on bone marrow samples sent by express airmail from El Salvador to SJCRH and using this information in diagnosis and risk assessment was also explored.

The project was initiated by a Salvadoran mother of a child acute myeloid leukaemia (AML) that was treated at SJCRH in Memphis, but died. She raised some funds along with some other volunteers and they then approached SJCRH to help establish a paediatric cancer centre at the Benjamin Bloom Hospital, San Salvador. A local paediatric haematologist was in charge of the programme and he, along with other health care providers received additional training at SJCRH. A paediatric-oncologist from SJCRH, who was fluent in Spanish, provided telephone and e-mail advice. Morphology and cytochemistry were performed locally. Bone marrow samples were also sent via 2-3 day courier to SJCRH for immunophenotyping, DNA content analysis and molecular genetics. Patients received a uniform remission induction regimen and were then stratified into standard and high risk groups for further treatment – the El Salvador I (ELS-I) treatment protocol.

Only 40% of the 401 children admitted with ALL to the Benjamin Bloom hospital between 1987 and 1993 received treatment and virtually none were cured (Ribeiro *et al*, 1996). Between 1994 and 1996 153 patients were enrolled into the ELS-I study. Of 151 evaluable patients 126 (83.4%) completed the induction regimen and went into remission, 11 (7.3%) were withdrawn by their families from treatment during induction and 14 (9.3%) were unable to complete the induction regimen due to complications. Of the 126 patients that went into remission, by 2001, 28 had relapsed, 5 had developed secondary AML and 22 had discontinued treatment during maintenance (Greenberg *et al*, 2001).

The 4 year EFS estimates for patients at high risk and standard risk were 46±7% and 69±15% respectively (Bonilla *et al*, 2000). Immunophenotyping was adequately performed in 127 patients (83%), DNA index was adequately performed in 123 (80%) and reverse transcription PCR was successful in 73 cases (48%). Problems encountered with the remote analysis of samples included the inability to get repeat samples when results were inconclusive as patients had already started treatment, loss of samples during in transit and delays in transit resulting in samples that were inadequate for testing.

As the number of samples increased remote testing became inefficient and costly and as a result an experienced Guatemalan pathologist was trained in flow cytometric analysis at SJCRH. Immunophenotyping and DNA content analysis is now being performed locally with SJCRH providing quality control and ongoing training. This transfer of techniques is crucial in the set up of a long-term treatment programme as described by Howard *et al* (2005). Another important aspect of this project was the level of local involvement. The project was initiated by a local group and was directed by an enthusiastic local clinician. Some of the funding for the project was also raised by local fundraising.

Lessons learnt from the ELS-I study have allowed the protocol to be modified and the project has been extended to Honduras and Guatemala as the El Salvador, Guatemala, Honduras-II trial (Greenberg *et al*, 2001).

Benefits of collaborations between developed and developing countries

Collaborations between health care workers and scientists in developed and developing countries are an excellent way to attempt to bridge the inequalities in health care and diagnostic techniques that exist between these countries. Such collaborations can be mutually beneficial – those in developing countries benefiting in terms of access to new techniques, resources to help improve patient management and most importantly training of staff to help improve patient diagnosis and care as well as staff morale. The benefits for those from developed countries involved in such collaborations include insight into different aspects of the disease in question as well as a reassessment of what is essential and what can and must be adapted and streamlined (Masera *et al*, 1998). Furthermore,

establishment of cancer treatment units in developing countries will attract more physicians and researchers and provide more information about the clinical, biological and epidemiological aspects of the disease in question (Greenberg *et al*, 2001).

There is no published data regarding similar collaborations to diagnose and treat LPDs in adults in resource poor countries.

Key elements in the development of a diagnostic and therapeutic service as a collaboration between developed and developing countries

Eden (2002) looked at the feasibility of translating a cure for ALL to all children and outlines the key elements to the successful development of a service in a disadvantaged country as follows:

- Recognition of a need in the centre/country itself.
- Mobilization of the local community (especially parents and families) to raise funds, put pressure on medical services, administrators and politicians to recognize the need and provide support, moral and ideally financial. The priority is to get attention and start to activate priority setting by authorities.
- Find an external partner unit which is already established and willing to help but will not dictate what is to happen in the country (or the unit), i.e. like the St Jude or La Mascota programmes.
- Development of medical, nursing and paramedical expertise in the diseases to be treated – this can often be done first by visiting fellowships whereby a short stay in an established unit in an industrialized country will provide specific expertise but in the long run must be developed in house or at least in country. Both the St Jude and Italian outreach programmes have excelled in this but also developed regional programmes bringing together countries with similar cultures and languages. The initiation of long-term recruitment, training, accreditation and retention of trained staff is important.
- Improvement of supportive care facilities, especially protection from those with infectious diseases and development of a safe and reliable blood transfusion service.

- Development of subsidised travel, home from home facilities, etc. to lessen the burden on parents.
- Development of appropriate protocols for each disease entity: these must be locally practicable with minimum cost and maximum efficacy.
- The process can be greatly enhanced by the formation of a co-operative group bringing together all the professionals involved in the speciality within a country or region to share expertise and develop training programmes.
- Such a co-operative group should act as an advocate for children with cancer in collaboration with civic groups and parent support groups, in order to lobby for the optimal care for children with life threatening conditions.

Collaborative diagnosis of LPDs in developing countries

The ILSG looked at 'the clinical characteristics and pathological classification of NHL in developing countries' and reviewed 206 cases of NHL diagnosed at the Kuwait Cancer Control Centre (Temmim *et al*, 2004). Over a period of two years patients diagnosed with NHL were entered into the study. Clinical information was documented, a diagnosis was made by local pathologists and a matching tissue block was sent to the ILSG referral centre in Germany. Diagnosis was made according to the WHO classification of haematological neoplasms and included routine staining as well as immunophenotyping using B- and T-cell markers (Jaffe *et al*, 2001). Five ILSG pathologists reviewed the blocks sent to Germany using the same panel of markers. Consensus diagnoses were made by the panel, first with limited clinical information and routine stains, then with the addition of immunophenotyping results and finally with full clinical information. In only 4 cases did the ILSG panel come to a diagnosis that differed from the local pathologists. In a further 4 cases the block that was sent to Germany was felt to be of too poor quality for a diagnosis to be made. The authors conclude that the WHO classification of haematological neoplasms is fully reproducible in their setting (Temmim *et al*, 2004). This project shows that remote comprehensive lymphoma diagnosis is feasible, although the ILSG panel diagnosis was made retrospectively as facilities existed locally for routine histology as well as immunophenotyping.

Summary

- With the increasing availability of chemotherapy and radiotherapy in Ghana, and other developing countries, there is an urgent need to improve the diagnosis of LPDs, and other malignancies, to ensure that appropriate treatment is given
- There is evidence that the spectrum of LPDs in Ghana, and other West African countries, is likely to be different to that found elsewhere
- The role of infection in the aetiology of LPDs in Ghana requires further investigation as this is likely to influence treatment options
- Real-time collaborative diagnosis alongside research, with a view to transfer of diagnostic skills and technology, would address these problems in a practical and sustainable manner

Chapter 2 – Setting up the Service: Process and Challenges

Introduction

The project's first objective was to set up a collaborative model between two teaching hospitals in Ghana and HMDS, Leeds for the diagnosis and classification of LPDs. This chapter describes how this was done and some of the challenges that were addressed in doing so.

The diagnosis of patients with a suspected LPD before the project was set up

Patients with suspected LPDs are referred to the haematology outpatient clinics at KATH and KBTH. Patients are referred by other specialists within the respective teaching hospitals, doctors working in the regional, district and other hospitals and those working in health centres and clinics. There are no set criteria for referral – this is at the discretion of the referring clinician, but includes leucocytosis, abdominal mass and lymphadenopathy. Patients with a suspected LPD are seen, investigated and treated according to the availability of tests and treatment and what the patient is able to afford, at the discretion of the clinician involved.

At KATH these patients are seen by the two specialists in the department of medicine with an interest in haematology, one also has an interest in oncology. Oncology clinics are held on Monday mornings and haematology clinics on Wednesday mornings. Although there is not a department of haematology at KATH, out patient clinics are held, and chemotherapy given, in a new, purpose built, radiotherapy building. In-patient care takes place on the general medical wards under the care of one of the five medical teams, but is avoided where possible as isolation facilities are not available.

Prior to the project commencing KATH did not have a functioning histopathology department and so biopsies had to be sent to one of the private laboratories in the town

centre from where they were sent to laboratories in Accra to be processed and reported. The oncology specialist performed and reported FNAs. BMAs were performed and reported by both haematology specialists, who also reported blood films. Bone marrow trephines were rarely done as they had to be sent to the private laboratories for processing and reporting.

KBTH, in contrast to KATH, has a haematology department as well as a functioning histopathology department. Within the haematology department there are 3 haematologists - a head of department and two retired professors that continue to work in the department. There are also several haematology residents working in the department as part of the pathology rotation. Residents take the West African College of Pathology exam prior to progressing to become specialists in their chosen area of pathology. BMAs, and occasionally trephines, are done by the haematology residents on Tuesdays and Thursday mornings in the haematology day unit. There is a direct referral system for bone marrows by clinicians from KBTH and other hospitals and so patients are often not referred to, or seen by, a haematologist for advice prior to their bone marrow.

Audit of patients seen at KATH in 2004 with a suspected diagnosis of LPD

In order to document how patients with a suspected LPD were investigated and treated prior to the project being set up, Dr Stephens audited the folders of patients presenting with a suspected LPD to the haematology and oncology clinics at KATH in 2004. Patients were identified by going through the clinic log books for 2004 and selecting patients where a provisional diagnosis of HMS, lymphoma, leukaemia, myeloma, splenomegaly or lymphadenopathy was entered and where, on review of the folder the patient would have fulfilled the criteria for entering the project. (Figure 2.1)

Figure 2.1 – Criteria for entry into the project

The criteria for project entry were: consenting patients, over the age of 12 years, with a suspected LPD. Whether a patient was suspected to have a LPD was at the discretion of the entering clinician, but relevant symptoms and signs included B symptoms (fever, weight loss and sweats), lymphadenopathy, hepatomegaly, splenomegaly, lymphocytosis, hypercalcaemia and lytic bony lesions. Patients with splenomegaly alone were entered if they had another suspicious feature or had failed to respond to three months of treatment for HMS with proguanil.

At KATH, patients have a separate folder for each hospital department and those for the haematology and oncology clinics are held in the Radiotherapy building. Only 63/80 folders were available however and the clinic log book did not appear to be complete as there was a large variation in the number of entries per clinic and some clinics were missing.

It was not possible to audit the folders for the KBTH patients as outpatient clinics were held in the general outpatient area and there was no record of attendees with diagnoses. Each patient also only had one folder for all the specialties within the hospital and these were held in a central records area and were not accessible.

In 2004 63 patients were seen at KATH fulfilling the above criteria. 21 were male and 42 were female. The median age was 48 years (range 12-80 years). 45 were seen in the haematology clinic and 18 in the oncology clinic. B symptoms (fever, sweats, significant weight loss) were documented in 26 patients (41%) at presentation, but in 26 out of 63 patients (41%) there was no mention in the folder regarding the presence or absence of B symptoms. At presentation 44 patients (69%) had splenomegaly (median length below the costophrenic angle 16cm, range 4-26cm), 27 patients (42%) had hepatomegaly (median length 6cm, range 3-16cm), 18 patients (28%) had lymphadenopathy and 5 patients (8%) had masses elsewhere in the body.

61 patients (87%) had a full blood count (FBC) and 31 (47%) had a blood film performed as part of their initial investigation. 1 patient was found to be HIV positive, 1 HIV negative, but the HIV status of the remaining 61 patients was unknown. FNAs were performed in 11 patients and 5 had a biopsy (3 lymph nodes, 1 stomach and 1 tonsil). 1 patient had both an FNA and a biopsy and 1 patient had a biopsy, but the result was not available. 1 patient had a BMA performed. This had to be repeated as the first sample was non-diagnostic. The results of the FNAs, biopsies and BMA, as well as the diagnosis after investigations, are shown in table 2.1. 43 patients (68%) were lost to follow-up, 37 (59%) within the first 6 months. 20 patients (32%) remained under follow-up in clinic, of which 4 had defaulted for a period of greater than 6 months during their follow-up.

Table 2.1 – 2004 Audit results - Diagnoses and how they were made

Diagnosis	Number of patients with diagnosis	Diagnosis made clinically	Diagnosis made on FNA	Diagnosis made on biopsy	Diagnosis made by BMA
HMS	28	28			
NHL	13	6	5	2	
HL	5		3	2	
LPD	5	5*			
CLL	5	5			
Myeloma/ plasmacytoma	4	2	1		1
ALL	1	1			
Portal hypertension	1	1			
Metastatic malignancy	1		1		
Total	63	48	10	4	1

*1 had had a biopsy, but result was not available

There was a striking male to female ratio (1:2), relatively young age of patients at presentation (median age 48 years) and a high default rate from follow-up (59% within the first 6 months). Splenomegaly was much more common (69% of patients) than one would expect to see in the UK in unselected new patients with a suspected LPD. B symptoms (41%) and hepatomegaly (42%) were also more common than seen in the UK. Virtually all diagnoses were made either clinically or following suboptimal investigation – BMA and FNA cytology.

Purpose of the project

The purpose of the project was to set up a system to diagnose and categorise LPDs in Ghana using modern diagnostic techniques, not currently available locally, in a clinically meaningful way. Results would be used to inform treatment decisions, as well as provide epidemiological data regarding LPDs in West Africa. This system would be robust and sustainable so that local clinicians could continue to use it once the initial project had finished and, as resources became available locally, transfer of skills and techniques was envisaged so that they could be performed locally.

How we developed the model we used

The collaboration

The project was a collaboration between:
The Haematology Specialists at KATH, Kumasi;
The Head of the Department of Haematology and one of the Haematology Residents at KBTH, Accra;
Dr Bates, Consultant Haematologist based at LSTM, Liverpool;
Dr Owen, Consultant Haematopathologist, HMDS, Leeds;
Dr Stephens, Haematology Specialist Registrar on the Leeds rotation, MD author.

Dr Bates spent 4 years working at KATH whilst she was a haematology registrar. She worked with one of the haematology specialists, as described in chapter 1, investigating

patients with splenomegaly and proposed a link between malaria, HMS and a low-grade LPD which they described as TSL. Due to a lack of locally available tests they were unable to fully immunophenotype or genotype these cases. They presented their work at HMDS in 2001. As a result of this presentation Dr Owen became involved in the collaboration. The Head of the Department of Haematology at KBTH had also expressed an interest in becoming involved in further work investigating Ghanaian patients with suspected LPDs and had previously presented work on LPDs at international haematological meetings.

Background about HMDS

HMDS was formed in 1993 by the amalgamation of the 'Leukaemia Diagnosis Unit' (which had itself been established in 1983) and the Yorkshire Regional Cancer Organisation Histopathology Laboratory. It is a fully integrated laboratory bringing almost all aspects of haematological malignant diagnostics together including flow cytometry, histology and molecular genetics. Scientists work alongside haematopathologists and results are reviewed by two senior scientists or consultants before being authorised.

All biopsy samples on patients suspected to have a haematological malignancy are sent to HMDS from the Yorkshire and Humberside Cancer Networks covering a population of four million. This includes lymph node biopsies, bone marrow aspirates and trephines, CSF, pleural aspirates and skin biopsies. Results are e-mailed to clinicians, available via a password protected website, sent out by post and discussed at multi-disciplinary team meetings throughout the region. HMDS is primarily a diagnostic service, but also has a strong research base, especially in the field of LPDs.

Planning trips to set-up the project

Dr Owen visited KATH and KBTH with Dr Bates in November 2003 to discuss the possibility of carrying out a collaborative project and meet local clinicians. Dr Stephens also visited with Dr Bates for a week in March 2004 and met the collaborators as well and the Head of the Immunology Department at the Noguchi Memorial Institute for

Medical Research (NMIMR). Plans for the project were discussed although the timing and funding of the project had not been finalized at that stage. A haematology resident spent three weeks at HMDS in June 2004 learning about diagnostic techniques such as flow cytometry and FISH.

Funding applications

Dr Stephens applied for funding for the project from the British Society of Haematology (BSH), the Leukaemia Research Fund, the Elimination of Leukaemia Fund, the LSTM Research and Development Fund and the Kay Kendall Fund, but was unsuccessful. Dr Stephens received a BSH travel grant to fund her planning trip and further funding for travel and consumables was received from the Myre Sim Fund and the Jean Clayton Funds via the Royal College of Physicians Edinburgh and LSTM respectively. Unrestricted educational grants were also received from Roche, Chugai-Pharma and Amgen. MD fees were paid by Dr Stephens.

The main problem encountered when applying for funding was that the project fell between funding for cancer research – for which the grants are for UK based projects, and funding for tropical research projects – for which the grants are mainly for preventable diseases and not for cancer-related research projects. It was possible to carry out the project, however, as Dr Stephens was employed as a lecturer at the Kwame Nkrumah University of Science and Technology (KNUST) Kumasi and no charge was made for sample processing and reporting at HMDS.

Ethical approval

The project was approved by the ethical committees of LSTM, KATH and the University of Ghana, Accra. As the patients were not subjected to any procedures other than those that form part of the usual investigation of patients suspected to have a LPD there were no significant ethical issues raised. The University of Ghana ethics committee required a more detailed consent form than that used at KATH. (Appendices 4 and 5) The KBTH consent form was therefore submitted and approved as an addendum by the LSTM ethics committee.

Laboratory training prior to the project commencing

Prior to the project commencing, Dr Stephens was taught how to prepare samples for flow cytometry, acquire and analyse data. Further telephone and e-mail support was provided by scientists at HMDS when flow cytometry was attempted in Ghana as well as assistance preparing and analysing samples when they were brought back to the UK. Training in other laboratory aspects was also given such as processing and embedding trephines and lymph node biopsies, immunohistochemistry, PCR and FISH.

Internet and postal services

In recent years there have been major advances in internet availability worldwide. In Ghana most towns have an internet café and whilst the project was taking place a hospital network system was installed at KATH. Before this happened there was a dial-up internet connection in the Department of Medicine library. This meant that the collaborators were able to stay in regular contact and that results were rapidly and reliably e-mailed back to Ghana. Samples were sent to the UK via United Postal Services (UPS). Each package weighing less than 500g cost 341 500 cedis (£21.34) to post and arrived at HMDS within 3-4 working days. All 25 parcels sent arrived intact and so this was a reliable and cost effective service. Alternative carriers are available at a similar cost – for example DHL, but we did not investigate this further as we had no problems with UPS. There are also companies that are able to transport samples on dry ice, but this is more complicated and much more expensive and was not necessary for our project.

Sourcing consumables

Where possible, consumables were sourced locally in Ghana. Other consumables were donated by HMDS. Needles, syringes, gauze, tape, lignocaine, skin preparation liquid, distilled water and gloves (both sterile and non-sterile) were readily available at both KATH and KBTH. Ethylenediamine tetra-acetic acid (EDTA) tubes, serum tubes, slides, phosphate buffered solution (PBS) powder, and ammonium chloride (NH_4Cl) powder were sourced from local suppliers via laboratory staff.

Formalin pots were initially donated by HMDS, but formalin was subsequently obtained from the medical school anatomy department KNUST and syringed into eppendorfs for bone marrow trephines. Larger pots were used for lymph node biopsies. For lymph node biopsies at KATH some surgical implements were available in the department, others were bought from local surgical suppliers as were sutures. Eppendorfs, parafilm, test tubes with caps, pastettes and antibodies for flow cytometry were all donated by HMDS as it was not possible to source these locally in Ghana. (Appendix 6)

Bone marrow needles

Reusable bone marrow aspirate and trephine needles were in use at both KATH and KBTH prior to the project commencing. The needles at both hospitals were relatively old and had become quite blunt. They were washed and sterilised between patients. We investigated sourcing disposable aspirate and trephine needles, but at a cost of approximately £5 per disposable aspirate needle and £10 per disposable trephine needle (despite a 30% discount), this was too expensive to be sustainable. A box of discontinued, disposable, trephine needles were donated by one company, but it was not possible to obtain donations of any further needles, either disposable or reusable, from any other companies. We were, however, given approximately twenty pairs of reusable aspirate and trephine needles by haematologists from several UK hospitals. Some of these had never been used having been brought just prior to the switch from reusable to disposable needles. Most hospitals in the UK moved over from using reusable needles to disposable needles in the 1990s.

Access to laboratory space and equipment

In order to process the samples for freezing, laboratory bench space, a centrifuge and a water bath or incubator at 37°C were required. At KATH the water bath in the haematology laboratory was used along with bench space and a centrifuge in the Department of Medicine and the freezer in the Head of Department's office. Unfortunately the water bath was therefore on the ground floor and the rest of the processing was on the third floor. At KBTH laboratory space with an incubator,

centrifuge and freezer was available in a room in the haematology department as well a laboratory technician who processed the samples.

Setting up and running the LPD clinic at KATH

As mentioned earlier the haematology clinic at KATH was held on Wednesday mornings and an oncology clinic with FNA service was held on Monday mornings. On arrival at KATH a second clinic room was prepared so that Dr Stephens could also take part in the haematology clinic on Wednesday mornings and enter relevant patients into the study. Dr Stephens also started a LPD clinic on Monday mornings and, as necessary, on Friday mornings so that patients from the oncology clinic and ward could be entered into the project. Bone marrows and lymph node biopsies were performed on the couch in this room and a trolley was set up to store the bone marrow equipment.

Patient entry

From the 1st March 2005 at KATH and 1st July 2005 at KBTH, consenting patients, aged ≥12 years, presenting to the haematology departments with a suspected LPD were included. The entering clinician decided if the patient was eligible for inclusion. (Figure 2.1) Patients were entered by the haematology resident in Accra and Dr Stephens, although patients were highlighted for entry by the other local collaborators.

The project was discussed with eligible patients, via an interpreter where necessary, using the appropriate project consent form and if they consented the patient signed or thumbprinted the form. (Appendices 4 and 5) If the patient was under 16 years of age a parent or guardian also signed or thumbprinted the form. A project pro forma for that patient was commenced and completed in as much detail as possible when the patient entered the project and outstanding results were completed as they became available. (Appendix 7) The signed consent form was stapled to the front of the patient's pro forma. When HMDS results were e-mailed back to Ghana two copies were printed off – one was filed in the patient's folder and the other was stapled to the back of the patient's pro forma. As the forms contained clinical information and the patient was identifiable by

name, hospital number and date of birth, they were kept in a locked drawer and a locked cupboard at KATH and KBTH respectively. Only the project clinicians had access to these forms.

Appropriate blood tests for the presenting symptoms were requested, for example, FBC with differential (dif) and film, lactate dehydrogenase (LDH), urea, creatinine and electrolytes, liver function tests, calcium, erythrocyte sedimentation rate (ESR). Appropriate scans and x-rays were also booked. If the patient had lymph nodes that were amenable to biopsy this was booked.

At six months and twelve months after entry into the project, clinical details and response to treatment were documented along with the repeat full blood count plus differential.

Database

Data was entered by Dr Stephens onto a password protected excel spreadsheet on her laptop. Patients were allocated a project number and this was used to enter their data onto the database. Patient name and hospital number were not entered onto the database to preserve anonymity. A single paper copy of the patient name against project number was kept.

Bone marrow and lymph node results were added to the database as soon as they were available as were the six and twelve month follow-ups. From the outset the database was set up to facilitate studying the results utilising code where necessary. The laptop was backed up weekly. Back up CDs were kept at two separate sites.

Sample collection

Blood and bone marrow collection

Samples were collected in the outpatient department at KATH and in the haematology day unit at KBTH, unless the patient was an inpatient and too unwell to leave the ward.

Samples for flow cytometry need to be analysed within 48 hours of being taken. This therefore dictated the timing of marrows when flow cytometry was being attempted locally. When flow cytometry was performed at HMDS samples were taken at KATH on Wednesday morning, at KBTH on Thursday morning and were processed at HMDS on Friday morning. This gave satisfactory results.

Approximately 6mls of PB was taken – 4 slides were made, 4mls was put into an EDTA tube and 2mls into a serum tube. Bone marrow aspirate and trephine were performed. Approximately 2mls of BMA was taken. 4slides were made for the project; others were made for local reporting. The remaining sample was put into an EDTA tube. The trephine sample was put into an eppendorf containing formalin.

Lymph nodes

At KATH lymph node biopsies were performed in the haematology outpatient clinic by one of the surgical residents. The node was sliced into approximately 2-3 millimetre thick slices to allow fixation by formalin. The lymph node sample was sent to HMDS via UPS.

If the patient had had a lymph node biopsy reported by one of the private laboratories prior to entry into the project, the laboratory was approached to ask if they would release a block for immunohistochemistry. A system was already in place at KBTH for referring patients with lymphadenopathy for biopsy which was then processed and reported by the hospital pathologists. Unfortunately it was not possible to obtain the KBTH pathology department blocks.

Sample processing and storage

Slides were allowed to dry before being wrapped tightly in parafilm to ensure that there were no trapped air bubbles. Approximately 1ml of serum was transferred into a clean eppendorf using a pastette. 2mls of PB was washed twice with PBS and then transferred to an eppendorf. 2mls of PB and 2mls of BMA were lysed twice using NH_4Cl and then washed three times with PBS. The residual pellets were transferred to eppendorfs.

(Appendix 2) The wrapped slides and four eppendorfs per patient were kept in a minus 20°C freezer until they were transported, packaged in a cool box according to International Air Transport Association regulations, back to HMDS by Dr Stephens. At HMDS they were stored in the minus 20°C freezer.

Handing over the project

Dr Stephens was in Ghana from the beginning of January 2005 until the end of March 2006. The first three months were spent acclimatising and setting up the project. The final three months were spent continuing to enter patients, but also handing over the project to local colleagues to allow patient entry to continue.

A laboratory technician at KBTH, had been processing the samples for freezing there. The haematology resident at KBTH had been entering patients into the project. The trephines were sent to Kumasi by bus for Dr Stephens to post to the UK with the samples from KATH. Dr Stephens made project packs for patient entry at KBTH containing all the consumables necessary for one patient's samples to be processed and stored as well as a consent form and pro forma. Twenty five packs were made and empty bags were refilled in Kumasi and sent back to Accra. The haematology resident at KBTH took over entering the data onto the database and a new, password protected database was set up on their laptop for ongoing patient entry in Accra.

At KATH a laboratory technician was identified and trained to process the samples. One of the haematology specialists took over entering data onto the database and a new, password protected database was set up on his computer for ongoing patient entry in Kumasi. Extra consumables were brought over from HMDS and locally available consumables were stocked up. Funds to cover posting samples back to the UK, further consumables, photocopying and paying nurses, the surgeon and laboratory technician were available locally.

Problems encountered

Setting up the project in two centres

Although the medical system in Ghana is based on the UK system there are some differences and at times it was a challenge to adapt the project to suit local circumstances. For example platelet transfusions are not available at KATH and only occasionally available at KBTH and so this needed to be borne in mind when deciding about doing bone marrow trephines and lymph node biopsies on thrombocytopenic patients. As it had been so difficult to make a histological diagnosis at KATH some of the clinicians had become used to treating patients empirically without making a diagnosis first and so they needed to be encouraged to refer the patients for investigation before treating them.

Patients lost to follow-up

In the 2004 audit 59% of patients were lost to follow-up at 6 months. In the project 55% of patients were lost to follow-up at 6 months. On two occasions relatives returned to the clinic to let us know that the patient had died – both of these had aggressive disease and were receiving chemotherapy. Locally important reasons for failure to attend follow-up include the difficulty and cost of getting to the hospital for appointments, the cost of review appointments, lost wages for the patient or carer whilst attending the hospital, the cost of investigations and or medications, partial or complete resolution of symptoms. Patients, or their families, sometimes decided to switch from conventional to traditional medicine, which was also costly.

Communication problems

The official language in Ghana is English, but there are at least 75 local languages of which Twi is the most widely spoken. Around Accra Fanti and Ga are also commonly spoken as well as other languages so English is quite widely spoken as a language common to many. In the Ashanti region, including Kumasi, Twi is the main local language and is sometimes used in schools, so English is not as widely spoken as it is elsewhere in the country. Despite this most young people speak at least some English as

do older people who went to school. People that do not speak English sometimes brought a relative to clinic with them to act as an interpreter. If this did not happen a nurse or student would act as an interpreter. Sometimes this would work well, other times it would not – for example sometimes it was clear that the person interpreting was using their discretion and altering what was said.

Some parts of the pro forma presented more problems than others. Obtaining a medication history was usually very difficult. Many patients were able to say that they were on medications, but did not know what for; others were able to say that they were on medications for malaria or blood pressure, for example, but not which medication. Patients were asked how many times a year they got malaria; for some this was fairly easy to quantify, many patients said that they never got malaria, but for others it was a difficult question to answer. The words for fever and malaria are the same in Twi and as a result are indistinguishable by patients. In Ghana almost all diagnoses of malaria are made clinically and not confirmed by laboratory testing.

Patients unable to pay for tests and treatment

As patients had to pay for all investigations it was not possible to request all the tests one might wish to carry out, for example haematinics, Coombe's tests or tests to complete staging. Clinicians in developing countries therefore use their discretion when ordering investigations and have to bear in mind how much they feel the result will alter the patient's management and what they judge or know the patient can afford. There is therefore a greater reliance on clinical findings supplemented, where appropriate, with relevant investigations. On a couple of occasions when patients could not afford a test that would significantly contribute to their investigation this was paid for from project funds, also the cost of checking the LDH was covered by project funds in approximately 10 patients to assist calculating the IPI. At KATH a FBC cost 31 000 cedis (£1.93), renal function cost 45 000 cedis (£2.81) and LDH cost 20 000 cedis (£1.25). An abdominal US scan cost 45 000 cedis, a chest x-ray 30 000 cedis and a CT scan requiring contrast cost 1 million cedis (£62.50).

Patient's also had to pay a fee for each clinic appointment, although it was sometimes possible to arrange for this fee to be waived, for example if the patient had returned just to have a bone marrow done. Treatment also had to be adapted according to the patient's ability to pay.

Although patients have to pay for all their tests and treatment, the extended family system usually means that relatives will contribute to the cost. This works best for older members of society with large extended families. Often the younger patients struggled the most to pay for investigations and treatment, especially those living away from the family home in order to find work.

Concerns regarding the quality of test results

Although quality control measures were in place in the hospital laboratories and departments of pharmacy and radiology, problems occasionally arose. For example it became evident that all the LDH results were above the normal range – even those on healthy individuals such as the biochemistry laboratory staff and members of their families. This was investigated and rectified after discussion with the manufacturers of the machine. It was evident however that results and drugs from outside the hospital could not necessarily be relied upon, and were often more expensive than the in hospital tariff, so patients were encouraged to get their investigations done and buy their drugs within the hospital where possible.

Flow cytometry

As has been mentioned in the previous chapter immunophenotyping is crucial in the diagnosis of LPDs. The Immunology Department at NMIMR in Accra has a flow cytometer which is mostly used during the malaria season for research work looking at immune responses to malaria. Permission was granted by the Head of the Department of Immunology at NMIMR, to use the flow cytometer. Many problems were encountered however, including difficulties calibrating the cytometer and getting the settings correct, difficulties recording and transferring data as the printer and CD drive attached to the cytometer computer were broken and the computer was not linked to the internet. After

many attempts at correcting the settings it was decided to not continue with flow cytometry locally. Immunohistochemistry on the trephines and lymph nodes gave good results and where further information was necessary PB and BMA samples were taken fresh to HMDS for flow cytometry on trips back to the UK.

It is hoped that the preliminary results of this project, which clearly demonstrates the need for local provision of flow cytometry, will facilitate future applications for funding to support such a service in Ghana.

Poor documentation

Documentation in the clinical notes was frequently not as clear or as comprehensive as occurs in the UK, largely due to the extra time pressures that clinicians are under. This made retrospectively reviewing the notes difficult. Also there was not a systemic filing system so it was sometimes difficult to locate test results in the folders. There were no computerised results systems so if the paper result for a test was lost one would have to rely on the transcribed copy of the result written in the laboratory or radiology log book and occasionally this was not complete.

Summary

- A collaborative model was set up to comprehensively diagnose LPDs in Ghana
- Local clinicians and technicians were closely involved with the design and running of the project with a view to the model being continued when the initial MD project finished
- Full diagnostic work up was performed on samples sent to the UK and the results were available to clinicians in Ghana in a clinically relevant time span
- Transfer of skills and technology is envisaged over the coming years to improve the diagnostic facilities available locally

Chapter 3 – Patient Characteristics and Diagnoses

Introduction

The project's second objective was to diagnose and classify patients presenting with a suspected LPD to KATH and KBTH. In this chapter the characteristics and diagnoses of the patients entered into the project are presented and discussed.

Methods

As described in Chapter 1, LPD diagnostic techniques available in developed countries have advanced significantly in recent years. The diagnosis and classification of LPDs combines clinical details with morphology, immunophenotype (assessed by flow cytometry and/or immunohistochemistry) and genotype (assessed by conventional cytogenetics, FISH or PCR). (Figure 1.2, Chapter 1) The entry criteria for the project are described in Figure 2.1 (Chapter 2) and the methods that were used to diagnose the suspected LPDs are described in Chapter 1 and Appendix 1.

As described in Chapters 1 and 2, before the project commenced, many of these techniques were not available to clinicians diagnosing LPDs in Ghana. In Kumasi the diagnosis of a LPD was usually based on clinical details, basic blood tests and, where relevant, FNAs. Occasionally BMA, and very occasionally histology reported in private laboratories, were available. In Accra more information was available as more BMAs were performed and biopsy samples (trephines/lymph nodes) were processed and reported in the hospital pathology department.

When patients entered the project, all available clinical information as well as basic demographic and socioeconomic details were documented on patient pro formas. This information was then entered into a database along with HMDS results and, where possible, patient follow-up. The results are presented below.

Results

To allow comparison of the results the patients were divided, based on their final diagnosis (Table 3.8), into 4 groups. The patients with a LPD were divided into those with SMZL (n=18) and those with other LPDs (non-SMZL LPDs, n=43) as SMZL was the largest single category of LPD. The patients that did not have a LPD were divided into those with palpable splenomegaly, but a non-diagnostic marrow (SND, n=29) and those with other diagnoses (n=60).

Patients with splenomegaly were only entered into the project if they had another feature suggestive of a LPD, or if their spleen size had failed to respond to 3 months of proguanil. (Figure 2.1, Chapter 2) This was done to exclude most patients with splenomegaly due to HMS, however many of the SND group of patients may have had HMS and a second pathology causing lymphadenopathy or B symptoms.

As discussed in chapter 1 the criteria for the diagnosis of HMS includes splenomegaly of at least ten centimetres below the costal margin, a serum IgM concentration at least 2SD above the normal mean concentration for the area, a sustained response to malaria prophylaxis with a reduction in the size of the spleen of at least 40% and evidence of the polyclonal nature of the lymphocytes. We did not measure the serum IgM levels of the patients, but none of these SND patients had a clonal lymphoid population and in 25/29 (86%) patients the spleen was at least ten centimetres below the costal margin. Of the 29 patients with splenomegaly and a non-diagnostic marrow 27 (93%) had another feature suggestive of a LPD and 2 (7%) had been entered into the study as they had not responded to 3 months malaria prophylaxis with proguanil.

The 'other diagnoses' group includes the following diagnoses: AML, Refractory Anaemia with Excess Blasts, MGUS, tuberculosis, Rosai Dorfman, metastatic carcinomas, as well as patients with a reactive marrow, but no splenomegaly and patients with no, or an inadequate, trephine. (Table 3.7)

The results for the 4 groups were analysed to look for statistically significant differences between the groups. In the following tables the p value is given and where this is significant (<0.05) this is in bold. Also whether Kruskal Wallis Test (KW) (for ordinal data) or Pearson Chi-Square Test (PCS) (for nominal data) was used is indicated.

Patient demographics

150 patients were entered into the project - 71 male, 79 female. The median age was 46 years (range 12-83 years). 117 were entered at KATH and 33 were entered at KBTH. 100 patients were referred from within the 2 teaching hospitals, 50 from other hospitals or clinics. (Table 3.1)

Table 3.1 - Patient demographics

	All patients	**SMZL**	**Non-SMZL LPDs**	**SND**	**Other diagnoses**	**p**
Number	150	18	43	29	60	
Male:Female	71:79	4:14	21:22	13:16	33:27	0.26 PCS
Median age yrs (range)	46 (12-83)	64.5 (40-78)	58 (12-80)	35 (12-70)	33.5 (12-83)	**<0.0001** KW
Entered KATH: Entered KBTH	117:33	17:1	34:9	26:3	39:21	0.028 PCS
Referred within hosp:referred outside hosp	100:50	12:6	27:16	22:7	39:21	0.677 PCS

Socioeconomic data was also recorded and analysed. Statistically significant differences between the groups were found for residence (p=0.0001 KW), education (p=0.005 KW), roofing material on accommodation (p=0.013 KW) and access at home to a stove (p=0.006 PCS), a television (p=0.034) and electicity (p=0.033). (Figures 3.1-3.4) There

were no statistically significant differences for marital status, co-habitees, dependents, job and transport to the hospital. (Appendix 8)

Figure 3.1 – Residence

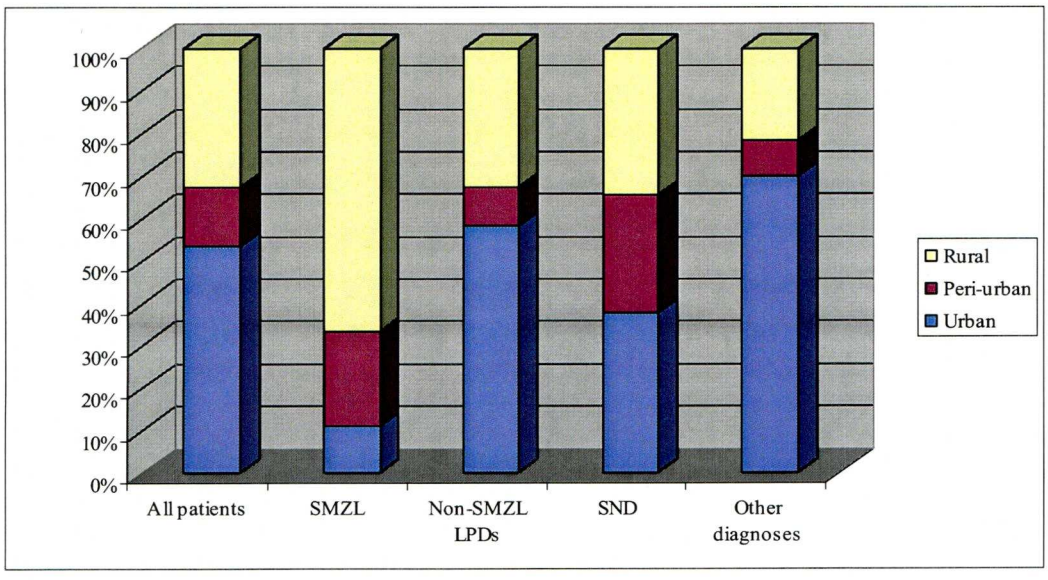


Figure 3.2 - Education

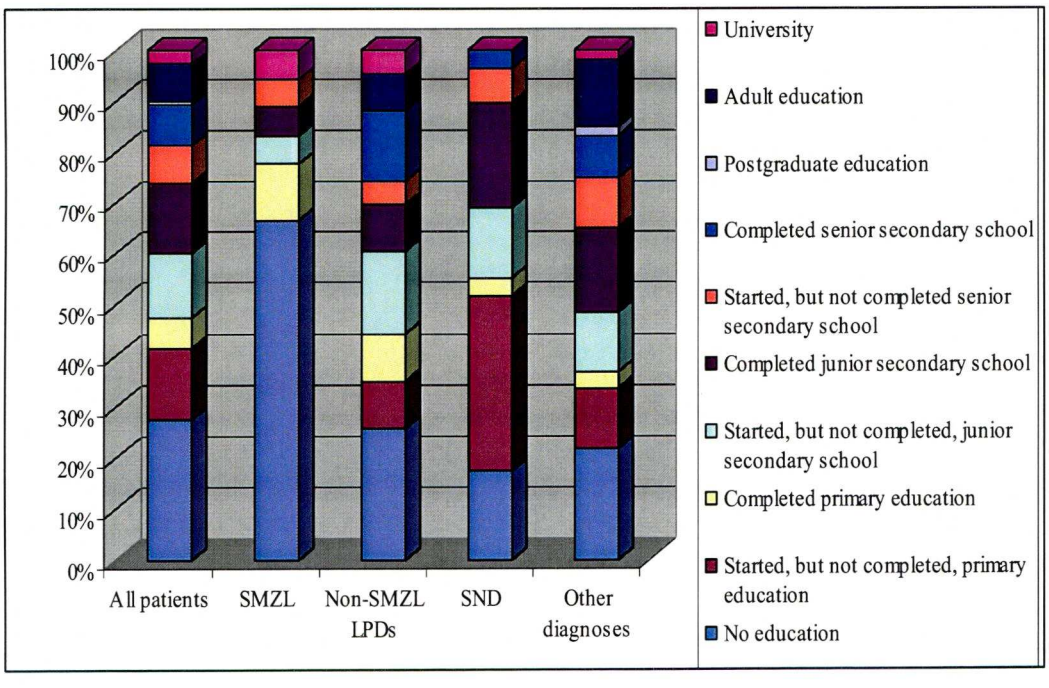


Figure 3.3 - Roofing material on accommodation

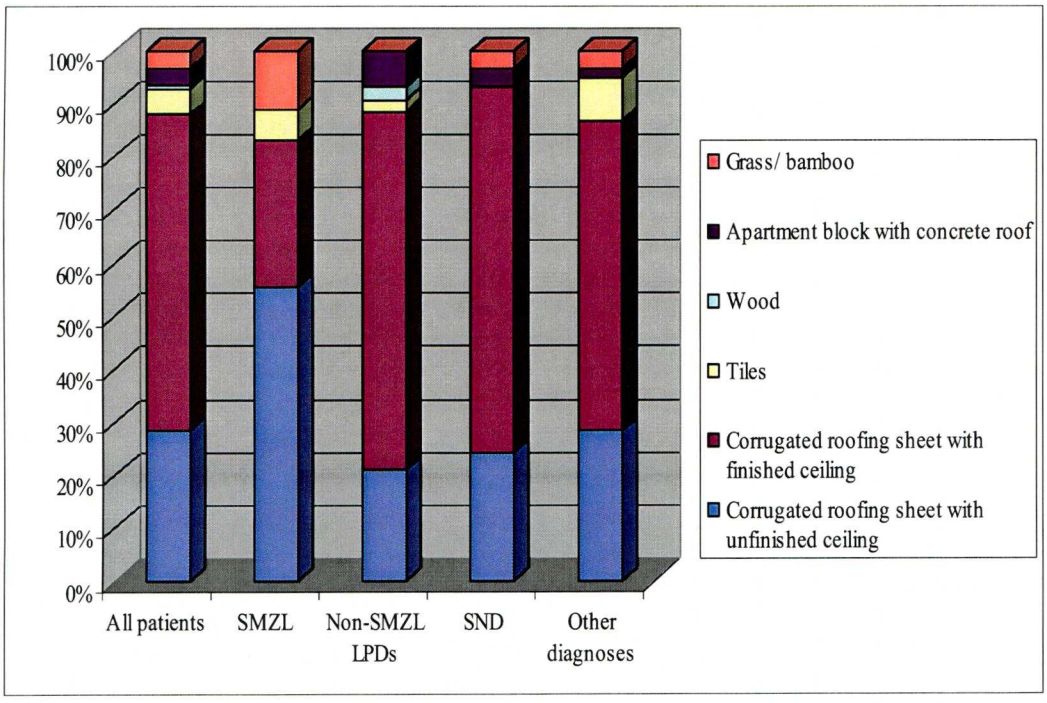
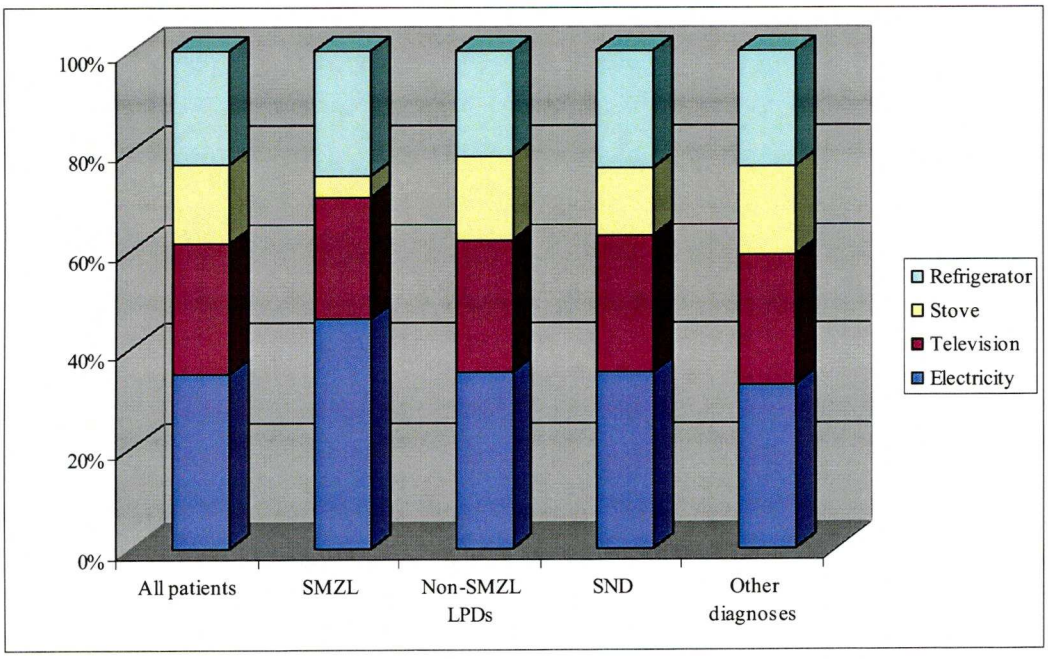


Figure 3.4 - Access to at home



Presenting symptoms

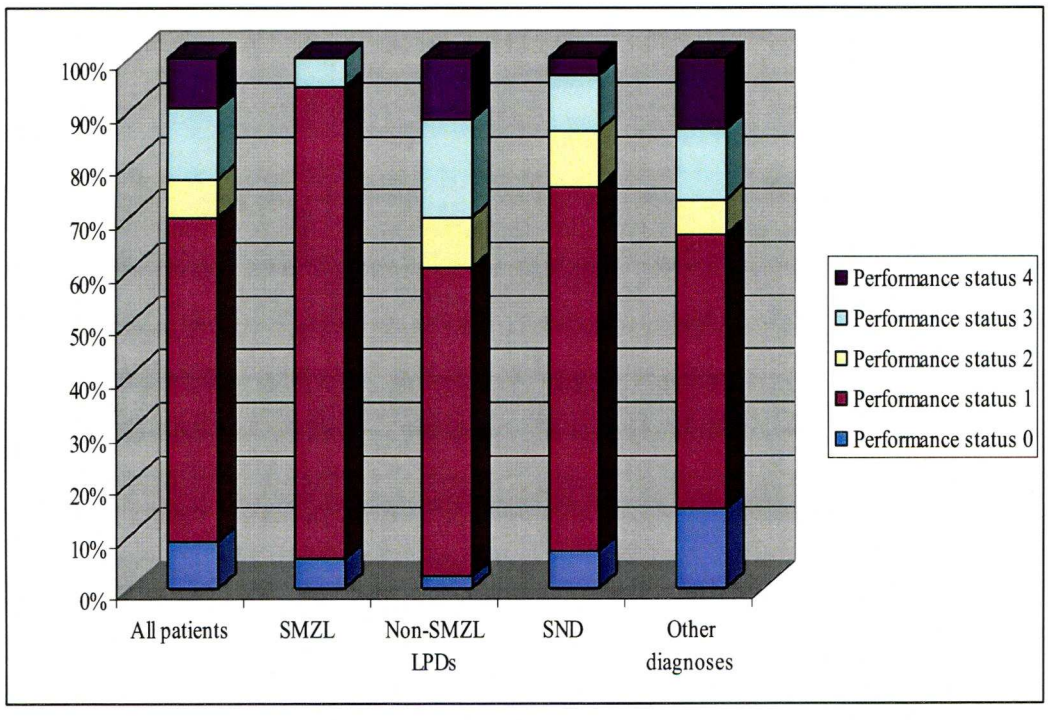
The presenting complaint was abdominal pain, distension or mass in 53/150 (35%) patients, lymphadenopathy in 32/150 (21%) and B symptoms (fevers, sweats or significant weight loss) in 20/150 (13%). The remaining 45 patients presented with other symptoms including fatigue, breathlessness, bone pain, headache and rash. On direct questioning at presentation 83/150 (55%) reported fevers, 78/150 (52%) reported sweats and 95/150 (63%) reported moderate-severe weight loss. Only 14/150 (9%) patients had no B symptoms when they were entered into the project. (Table 3.2)

Table 3.2 – Symptoms at presentation

	All patients n=150	**SMZL n=18**	**Non-SMZL LPDs n=43**	**SND n=29**	**Other diagnoses n=60**	**p**
Abdominal pain/ distension/mass	55 (37%)	14 (78%)	11 (26%)	17 (59%)	13 (22%)	**<0.0001** PCS
Symptomatic lymphadenopathy	32 (21%)	1 (6%)	13 (30%)	2 (7%)	16 (27%)	**0.010** PCS
On direct questioning reported fevers	83 (55%)	9 (50%)	24 (56%)	22 (76%)	28 (47%)	0.066 PCS
On direct questioning reported sweats	78 (52%)	12 (67%)	25 (53%)	15 (52%)	26 (43%)	0.298 PCS
On direct questioning reported significant weight loss	95 (63%)	11 (61%)	34 (72%)	17 (59%)	36 (60%)	0.533 PCS

13 (9%) patients were performance status 0 (asymptomatic), 91 (61%) patients were performance status 1 (symptoms, but ambulatory), 11 (7%) were performance status 2 (bedridden for less than half of each day), 20 (13%) were performance status 3 (bedridden for greater than half of each day) and 14 (9%) were performance status 4 (chronically bedridden). There was not a statistically significant difference regarding performance status between the 4 groups (p=0.195 KW). (Figure 3.5)

Figure 3.5 – Performance status of patients



Presenting signs

Clinical signs at presentation included palpable splenomegaly, hepatomegaly and lymphadenopathy. Splenomegaly was documented in centimetres palpable from the costophrenic angle and measured using a previously validated uniform technique to maintain consistency between clinicians (Bedu-Addo & Bates 2002). Other signs at presentation included pallor (22/150 patients), cachexia (6/150 patients) and jaundice (3/150 patients). (Table 3.3)

Table 3.3 – Presenting signs

	All patients n=150	**SMZL n=18**	**Non-SMZL LPDs n=43**	**SND n=29**	**Other diagnoses n=60**	**p**
Palpable splenomegaly	83 (55%)	18 (100%)	20 (47%)	29 (100%)	16 (27%)	
Size - median (range)	*13cm (2-33cm)*	*17cm (6-30cm)*	*11.5cm (3-25cm)*	*14cm (2-33cm)*	*6cm (2-31cm)*	**0.003** KW
Palpable hepatomegaly	60 (40%)	15 (83%)	15 (35%)	19 (66%)	11 (18%)	
Size - median (range)	*4cm (1-22cm)*	*5cm (2-17cm)*	*6cm (3-22cm)*	*4cm (2-7cm)*	*4cm (1-10cm)*	0.160 KW
Palpable nodes >1cm diameter	60 (40%)	3 (17%)	22 (51%)	9 (31%)	26 (43%)	**0.003** PCS

Medication and malaria history

When they were entered into the project 67% of patients were taking prescribed medications and 40% had had herbal medications in the previous six months. There was no significant difference regarding this between the four groups. The SMZL patients reported having had a median of 3 episodes of malaria (range 0-24) in the previous year. The group with SND and the group with 'other diagnoses' each reported a median of only 1 episode of malaria (range 0-20 and 0-24 respectively). This difference was of borderline statistical significant (p=0.067 KW).

Investigation results available locally at diagnosis

Almost all the patients (95%) had a FBC performed at diagnosis and most of these also had a blood film comment. Approximately half the patients had their renal function checked and half had a LDH. HIV test results were available in 43%, liver function in 37% and an ESR in 35%. There was no significant variation between the 4 groups in these frequencies. 33% of patients had an US scan, 16% an x-ray and 3% a CT scan, again with no significant variation between the 4 groups. There was a statistically

significant difference between the 4 groups regarding those that had an FNA reported locally, but not for those that had a biopsy reported locally (Table 3.4).

Table 3.4 – Investigation results that were available locally at diagnosis

	All patients n=150	**SMZL n=18**	**Non-SMZL LPDs n=43**	**SND n=29**	**Other diagnoses n=60**	**p**
Biopsy reported locally	16 (11%)	1 (6%)	6 (14%)	0	9 (15%)	0.052 PCS
FNA	21 (14%)	0 (0%)	6 (14%)	1 (3%)	14 (23%)	**0.017** PCS

There was a significant difference in the FBC results between the 4 groups, but no significant difference in the other blood results between the 4 groups. (Table 3.5)

Table 3.5 – The results of the blood tests that were available locally at diagnosis

	All patients n=150	**SMZL** n=18	**Non-SMZL LPDs** n=43	**SND** n=29	**Other diagnoses** n=60	**p**
Haemoglobin (g/dl) median (range)	8.6 (2.1-15)	8.5 (3.6-13.5)	8.2 (2.1-15)	7.2 (2.5-12.7)	10.4 (3.5-13.5)	**0.003** KW
WCC ($x10^9$/l) median (range)	7.5 (1.0-UR)	53.7 (2.8-UR)	10.8 (1.4-UR)	3.3 (1.0-26.1)	7.3 (2.0-264.0)	**<0.0001** KW
Platelets($x10^9$/l) median (range)	137 (3-682)	114 (14-181)	128 (6-518)	72 (6-317)	228 (3-682)	**<0.0001** KW
Creatinine (µmol/l) median (range)	88 (27-212)	97 (35-141)	88 (32-186)	62 (27-177)	88 (35-212)	0.221 KW
Bilirubin (µmol/l) median (range)	15 (3-125)	15 (10-125)	19 (7-34)	22 (8-56)	14 (3-109)	0.213 KW
Alanine tranaminase (U/l) median (range)	21 (10-334)	20 (12-56)	16 (11-334)	21 (11-47)	28 (10-80)	0.864 KW
ESR (mm/hr) median (range)	80 (4->140)	114 (16->140)	136 (4->140)	37 (12->140)	65 (15->140)	0.424 KW
LDH (U/l) median (range)	378 (91-5629)	424 (167-585)	372 (110-2735)	401 (122-1928)	356 (91-5629)	0.924 KW
Positive HIV test/number tested	7/65 (11%)	0/8 (0%)	1/21 (5%)	4/14 (29%)	2/22 (9%)	0.513 KW

Normal ranges: Haemoglobin 11.1-18 g/dl, WCC 2.5-10 $x10^9$/l, Platelets 140-440 $x10^9$/l, Creatinine 53-124 µmol/l, Bilirubin 3.4-22.2 µmol/l, Alanine tranaminase 10-40 U/l, ESR <20mm/hr, LDH 100-190 U/l. UR=unrecordable.

HMDS results

Most patients (88.7%) had a bone marrow aspirate and trephine reported at HMDS, 2 patients (1.3%) had just PB assessed at HMDS and 15 (10%) had neither PB nor bone marrow assessed at HMDS. The reasons for not having a bone marrow included the

patient being lost to follow-up or dying before the bone marrow was performed, being too thrombocytopenic for a trephine to be felt to be safe or patient preference. (Table 3.6)

The median turnaround time for bone marrow results from HMDS was 13 days (range 6-62 days) from when the sample was posted to the result being available to the clinician in Ghana and 15 days (range 7-62 days) from when the sample was taken to the result being available.

For biopsy results, the median turnaround time from HMDS was 10.5 days (range 3-27 days). This is the time from when the sample was posted to the result being available to the clinician in Ghana. Some of the biopsy samples were sent as blocks sometime after the biopsy had originally been taken and so the time from the sample being taken does not reflect the HMDS turnaround.

Table 3.6 – Samples processed at HMDS

	All patients n=150	**SMZL n=18**	**Non-SMZL LPDs n=43**	**SND n=29**	**Other diagnoses n=60**	**p**
Peripheral blood for flow cytometry, no bone marrow	2 (1.3%)	0	1 (23%)	0	1 (1.7%)	0.799 PCS
Bone marrow aspirate and trephine	133 (88.7%)	18 (100%)	42 (97.7%)	29 (100%)	44 (73%)	**<0.0001** PCS
Lymph node biopsy	28 (18.7%)	0	12 (27.9%)	2 (69%)	14 (23%)	**0.018** PCS

Breakdown by diagnosis

Table 3.8 shows the demographics of patients with the various diagnoses for comparison. The patients with LPDs will be discussed in greater detail in chapters 4 and 5 (cases 1 and 5). 3 patients with non-LPD diagnoses are presented in chapter 5 (cases 2-4).

Table 3.8 - Breakdown by diagnosis

Diagnosis	Number of patients with diagnosis Total=150	Age yrs median (range)	Sex M:F	% patients with B symptoms at presentation	% patients with a palpable spleen	WCC $x10^9/l$ median (range)
SMZL	18	64.5 (40-78)	4:14	94%	100%	53.7 (2.8-UR)
CLL	12	60.5 (45-80)	1:2	92%	67%	66.9 (4.1-UR)
HL	7	18 (14-69)	3:4	100%	71%	3.2 (1.4-21.9)
Myeloma	6	61 (50-74)	1:1	100%	17%	6.5 (3.6-10.8)
DLBL	4	55 (13-79)	1:1	80%	25%	4.7 (3.9-8.6)
T-ALL	3	25 (16-62)	2:1	100%	33%	20.7 (3-100)
Peripheral T cell lymphoma	3	30 (12-69)	3:0	67%	0%	6.9 (5.9-16.3)
B-ALL	2	30 (12-48)	1:1	100%	50%	33.8 (27.3-40.2)
FL	2	60.5 (56-65)	1:1	100%	50%	26 (7.7-44.3)
BL	2	33 (13-50)	1:1	100%	0%	7.7 (3.1-12.3)
MCL	1	70	1:0	100%	100%	UR
NK cell LPD	1	67	0:1	100%	100%	30.4
AML	4	25 (12-40)	1:1	75%	50%	129.2 (43.3-240)

Table 3.8 - Breakdown by diagnosis continued

Diagnosis	Number of patients with diagnosis Total=150	Age yrs median (range)	Sex M:F	% patients with B symptoms at presentation	% patients with a palpable spleen	WCC $x10^9/l$ median (range)
Refractory Anaemia with Excess Blasts	2	69 (69-70)	1:1	100%	50%	34.9 (9.2-60.6)
MGUS	1	65	0:1	100%	0%	unknown
Tuberculosis	4	32 (18-83)	3:1	75%	0%	9.7 (6.7-9.4)
Rosai Dorfman	1	30	1:0	100%	0%	5.3
Metastatic carcinoma	8	55 (18-78)	7:1	100%	13%	7.5 (2-18)
SND	29	35 (12-70)	13:16	93%	66%	3.3 (1-26.1)
Reactive marrow no splenomegaly	22	32 (13-77)	1:1	77%	0%	6.1 (3.6-49.1)
No/inadequate trephine	18	32.5 (12-64)	8:10	94%	71%	6.7 (3.6-241)

Comparison between initial and final diagnoses

The benefit (or not) of having diagnostic support for LPDs in Ghana from HMDS was determined by assessing whether the final pathological diagnosis altered patient management compared to the initial, largely clinical, diagnosis which was made based on locally available diagnostic tools. Patients were divided into 5 groups depending on the relationship between the initial and final diagnoses. (Table 3.7)

Table 3.7 – Initial compared to final diagnoses

Group (N=)	Initial diagnosis	Effect of final diagnosis on patient management
1 (48)	Initial and final diagnosis the same	No alteration in management
2 (23)	No firm initial diagnosis made; correct final diagnosis included in initial differential diagnosis	Final diagnosis informed management
3 (16)	Final diagnosis not included in initial differential diagnosis	Final diagnosis did not affect management
4 (23)	Final diagnosis not included in initial differential diagnosis	Final diagnosis informed management
5 (40)	Patient died, lost to follow-up or inadequate/ unobtainable sample	Final diagnosis did not affect management

Follow-up

Follow-up details were documented, where possible, when patients attended for clinic review at 6 and 12 months. Further follow-up was also planned at 18 months. Only 7 patients had been in the study for 12 months before the end of March 2006 and of these 4 (57%) were followed up; the other 3 (43%) patients had been lost to follow-up within 6 months of entering the project.

76 (51%) patients had been in the study for 6 months before the end of March 2006 and of these 33 (43%) had a 6 month follow-up. Of the remaining 43, 13 (17%) had died and 30 (40%) had been lost to follow-up. (Table 3.9) By the end of March 2006, of all 150 patients entered into the project, 21 (14%) had died and 35 (23%) had been lost to follow-up.

Table 3.9 – Follow-up at 6 months

	All patients	SMZL	Non-SMZL LPDs	SND	Other diagnoses	p
Eligible 6mth follow-up	76/150 (51%)	10/19 (53%)	21/43 (49%)	16/29 (55%)	29/59 (49%)	0.897 PCS
Followed up at 6 months	33/76 (43%)	5/19 (26%)	9/21 (43%)	9/17 (53%)	10/29 (35%)	0.431 PCS
Died before 6 month follow-up	13/76 (17%)	0/19 (0%)	6/21 (29%)	3/17 (18%)	4/29 (14%)	0.304 PCS

Discussion of results

Patient demographics

There was a significant difference between the 4 groups of patients in terms of age with the 2 groups of patients with LPDs being significantly older than the 2 groups of patients without a LPD (p=<0.0001 KW). As most LPDs, especially low grade LPDs such as SMZL, tend to occur in patients over the age of 50, this is not surprising. There was no significant difference in the sex ratios between the 4 groups, however the sex ratio in the SMZL group was striking and will be discussed further in chapter 4.

As Dr Stephens was based at KATH and patient entry to the project started earlier there, more patients were entered at KATH than at KBTH. Although it was not statistically significant, the proportion of patients entered at KATH versus KBTH is most striking for those presenting with big spleens – the SMZL and the SND groups. The work done by Dr Bates and others at KATH, investigating patients with big spleens had increased awareness amongst local clinicians, both at KATH and in local clinics and hospitals, thus

encouraging the referral of patients with splenomegaly to the haematology clinic. It is also not possible to exclude a real difference in the incidence of splenomegaly between Kumasi and Accra in a study of this size and design.

Most patients were referred from within the hospital as clinics tended to refer to the main outpatient areas, for example the polyclinic at KATH, where the doctors would do baseline investigations and refer patients on to specialist clinics as appropriate. The polyclinic also functioned as a primary care service for those patients living within the vicinity of the hospital as patients did not need a referral in order to be seen there.

The most striking and statistically significant differences in the socioeconomic details were the number of patients with SMZL that lived rurally, had no education and did not have access to a stove compared to the other groups. 'Stove' refers to a conventional oven inside the house; in Ghana those that do not have access to a stove do their cooking outdoors in a pot over a fire. These patients were therefore more likely to be susceptible to infections and less likely to receive prompt and through treatment of infection than those with more education, indoor cooking facilities and better access to healthcare facilities. This is potentially important when considering potential aetiological factors for SMZL, in particular malaria.

Presenting symptoms and signs

In just over a third of all patients, and in 78% of SMZL and 59% of SND patients, the presenting complaint was abdominal pain or mass – given the median palpable size of spleen in those that had splenomegaly (13cm, 17cm and 14 cm respectively) this is unsurprising. On direct questioning most patients (91%) reported the presence of B symptoms. There are several possible reasons for this including late presentation of LPD patients, patients presenting with infection as a cause of their symptoms and signs (e.g. those with tuberculosis) and patients presenting with an active infection and being found to have lymphadenopathy, organomegaly etc as an incidental finding.

Medication and malaria history

Two thirds of the patients reported that they were taking medications at presentation and two fifths that they had taken herbal medications within the past six months. This reflects the fact that patients were seen as secondary and tertiary referrals and that traditional medicine is still widely used in Ghana, even amongst those patients that can access medical services. We attempted to gauge how frequently patients were unwell with malaria – this was often a difficult question for the patients to answer and so must be interpreted with caution, but overall the SMZL group presented with a median of 3 episodes of malaria a year, 2 episodes in the other LPD group and 1 episode a year in the remaining groups. This was of borderline statistical significant difference.

Investigation results available locally at diagnosis

Of the results that were available locally at diagnosis the FBC results were the only results that were significantly different between the 4 groups, in particular for the WCC and the platelet count. The WCC was much higher in the SMZL group (median 53.7 $x10^9/l$) than in the other groups and the platelet count was lowest in the group with splenomegaly, but a non-diagnostic marrow. Interestingly the patients with splenomegaly, but a non-diagnostic marrow were more pancytopenic as a result of hypersplenism than the patients with SMZL who had both marrow infiltration and hypersplenism.

HMDS results

The project turn around time for results was very rapid especially compared to the local turn around time for histology samples processed in private laboratories which was at best a few weeks and in many cases several months. Rapid turnaround in the HMDS laboratory is facilitated by screening on sample arrival, so that the most appropriate investigations are performed in a timely manner along with resin embedding of trephine biopsies which is significantly shorter than standard decalcification and paraffin embedding. Samples are then reported by two haematopathologists. Results were e-mailed back to Ghana.

Breakdown by diagnosis

LPD diagnoses are discussed in detail in chapter 4. Of the non-LPD diagnoses eight metastatic carcinomas were diagnosed – of these five were diagnosed on bone marrow trephine reflecting late presentation of disease. The primary sites of these five cases were: one prostate carcinoma (the patient also had a lymph node biopsy showing prostate carcinoma), one probable gastrointestinal carcinoma and three adenocarcinomas of unknown primary (one patient also had a lymph node biopsy showing adenocarcinoma). Of the three carcinomas diagnosed on lymph node biopsy alone one was an EBV-related NPC, one was a poorly differentiated metastatic carcinoma and the third was a poorly differentiated carcinoma consistent with a lymphoepithelial carcinoma.

There were also four patients with AML and two with refractory anaemia with excess blasts; all six had peripheral blasts and so had been entered into the study as possible cases of ALL. Cytosine (the chemotherapy drug that is the mainstay of treatment for AML) was not available in Ghana, but vincristine, cyclophosphamide, doxorubicin and prednisolone (drugs used to treat ALL) were. Therefore whenever there was uncertainty regarding the lineage of blast cells it was assumed that they were lymphoid in origin and the patient offered a trial of vincristine and prednisolone, as AML was not treatable. As platelet transfusions, inpatient isolation facilities and drugs, such as growth factors and antifungals, were not available at KATH adult acute leukaemia patients had a very poor prognosis and generally died within a few days of diagnosis from bleeding or infection.

When it was possible to confirm the diagnosis of AML, for example if Auer rods were visible or a result was available from HMDS, this often raised an ethical dilemma for local clinicians. The concept of palliative care was not something that most Ghanaian families understood and therefore if a patient with an untreatable illness such as AML were allowed home to die the family would often resort to paying large amounts of money to the local healer in the hope of a miracle cure. As a result local clinicians, faced with a terminal untreatable illness tended not to discharge patients and rather allow them to die in hospital where they could attempt to alleviate their symptoms, although sadly even drugs such as morphine, were very costly.

Comparison between initial and final diagnoses

In almost a third of patients (n=48) the diagnosis made based on locally available information and the diagnosis made once the HMDS results were available were the same – 10 of these diagnoses were made on the clinical picture alone and 18 on the clinical picture plus blood film. This reflects the clinical acumen of local physicians who are used to having to make diagnoses and treat patients based on limited investigation results. In 15% of patients the HMDS diagnosis was part of the differential diagnosis made locally, but it would not have been possible to make to appropriate treatment decisions without the additional results. In a further 15% of patients the HMDS diagnosis had not been part of the initial differential diagnosis and this altered patient management – of these one third of the local diagnoses had been made based on FNA appearances.

FNA cytology alone is unreliable in the diagnosis of LPDs, but is a rapid, cheap and non-invasive investigation and therefore readily applicable to the developing country setting where patients often present late and cannot afford expensive investigations. As discussed in Chapter 1 both Patil & Bem (1993) and Bezabih & Mariam (2003) found FNA results were useful as a first line investigation of lymphadenopathy, but did not allow an accurate enough diagnosis for the correct chemotherapy or radiotherapy regimens to be chosen.

Where facilities for flow cytometry are available locally, immunophenotype results can give useful information in addition to FNA cytology (Jorgensen, 2005). Swart *et al* (2007) performed FNAs in 124 cases of suspected lymphoma in Capetown, South Africa and assessed the aspirate by both cytology and flow cytometry. Patients also had a biopsy and bone marrow aspirate and trephine and the FNA results were compared with the histological diagnoses. It was not possible to do this in 43 cases (35%) however as in 17 cases (13.7%) insufficient cells were aspirated for both cytological and immunophenotypic assessment and in 26 cases (21%) there was no confirmatory diagnosis.

In the 81 cases (65%) where it was possible to verify the FNA result with a biopsy result there were 13 true negative results (2 tuberculosis, 8 reactive, 3 granulomatous infection),

2 false negatives (one T-cell lymphoma and one FL diagnosed on biopsy) both of which were felt on review to be due to FNA sampling error and 2 false positives giving a sensitivity of 96.9%, specificity of 86.7%, positive predictive value of 96.9% and negative predictive value of 86.7%. In 64 cases (51.6%) an accurate diagnosis of lymphoma had been made on FNA. The authors did not give a full break down of the diagnoses, but report that of the 6 cases of HL, 4 were correctly diagnosed on cytology and 2 required biopsy. They also comment that it was easier to make a confident diagnosis of DLBL, anaplastic large cell lymphoma and SLL on FNA, and more challenging to diagnose MALT lymphoma and MCL. This is interesting as MCL is a LPD that could potentially be diagnosed on FNA if facilities were available for both flow cytometry and FISH looking for the t(11;14) translocation.

The facilities to perform flow cytometry are not available in most hospitals in sub-Saharan Africa and many do not have a pathologist expert in cytology reporting therefore it is not yet possible to use these techniques with this level of accuracy in most other sub-Saharan African countries. FNAs, even in expert hands with the addition of flow cytometry, remain insufficient for the diagnosis of LPDs and histological diagnosis remains crucial especially in HL and FL (Hehn *et al*, 2004). In the resource poor setting where there is a functioning histopathology service in place, it is cheaper and easier to introduce immunohistochemistry than flow cytometry, as the latter requires the purchase and ongoing maintenance of a flow cytometer. Where there is no functioning histopathology service in place then remote diagnosis of LPDs, such as in our project, needs to be considered.

Follow-up

39% of the patients that were eligible for 6 month follow-up had already been lost to follow-up. This is a common problem in developing countries and as discussed in Chapter 2 there are many reasons for patients not attending for follow-up.

Summary

- It was possible to remotely diagnose and classify patients presenting with a suspected LPD in a clinically relevant time scale
- The use of state of the art diagnostic techniques improved diagnosis and the management of many patients, even in a setting where there were limited therapeutic options
- 41% of patients with a suspected LPD had a LPD, 13% had other diagnoses and in 46% there remained some diagnostic uncertainty

Chapter4 - Lymphoproliferative Disorders

Introduction

The aetiology, diagnosis and classification of LPDs are discussed in Chapter 1. The way the project was set up is described in Chapter 2 and the laboratory methods used are described in Appendix 1. The patient details and characteristics of all the patients entered into the project are presented in Chapter 3. In total 61 (40%) patients in the project were diagnosed as having a LPD. This chapter describes these patients in greater detail and discusses relevant aspects of the LPDs diagnosed. Firstly the spectrum of LPDs diagnosed in the project will be compared to the spectrum of LPDs diagnosed at HMDS and then the diagnoses will be discussed in greater detail – SMZL, CLL, aggressive lymphomas and other LPDs.

Spectrum of LPDs diagnosed in project

61 LPDs were diagnosed in the project in Ghana between 1st March 2005 and 31st March 2006. Figures 4.1 and 4.2 show a comparison between these and the 1392 LPDs diagnosed in adult patients at HMDS between 1st April 2007 and 31st March 2008. There are several striking differences and some unexpected similarities between the 2 groups, although the numbers in the project were small. The 2 groups were compared using the Simple Proportions Test to look for statistically significant (p value of <0.05, highlighted in bold) differences between the proportions of patients with the different diagnoses in each group.

Firstly the proportion of cases of SMZL in the project population was more than 6 times that in the HMDS population (29.5% versus 4.7%, **$p<0.0001$**). This is despite a similar proportion of cases of CLL in the 2 populations (19.7% versus 28.4% respectively, $p=0.1355$). The HMDS results are consistent with those of Thieblemont *et al*, who

reported that 81 out of 3038 (2.7%) patients with NHL in Lyon, France had SMZL (2003).

Figure 4.1 – Spectrum of LPDs diagnosed in project (n=61)

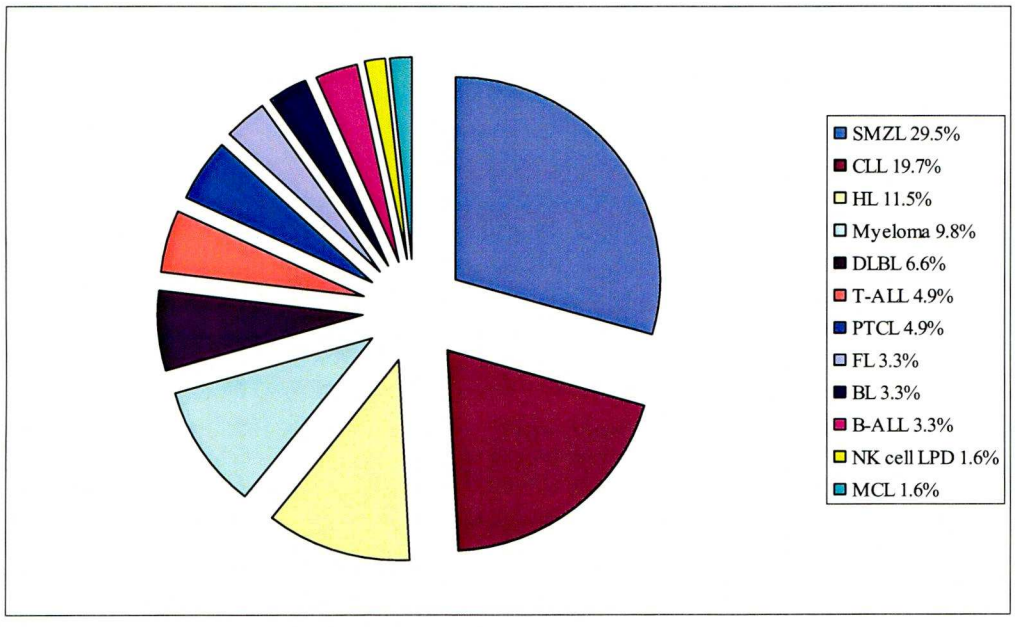
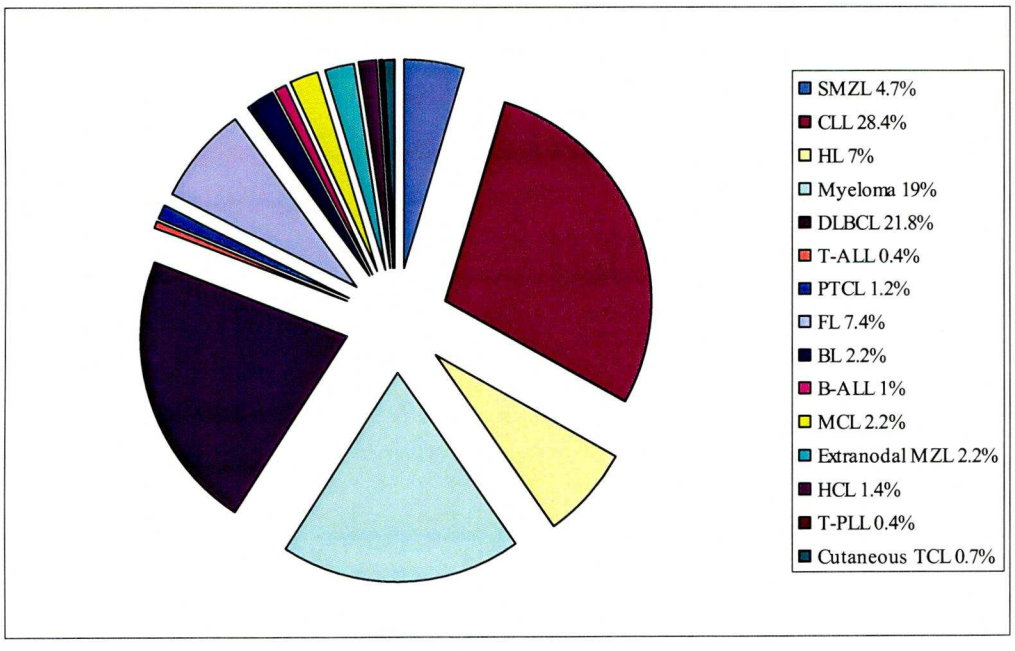


Figure 4.2 Spectrum of LPDs diagnosed at HMDS (n=1392)



HCL=Hairy cell leukaemia, PLL=Prolymphocytic leukaemia, TCL=T-cell lymphoma

Comparison of cases of DLBL also showed a striking difference between the two populations of patients with only 6.6% of the project population having DLBL compared with 21.8% of the HMDS population (**p=0.0043**). This is again despite similar proportions of HL being diagnosed in the two groups (11.5% and 7% respectively p=0.1814). The relative proportion of cases of DLBL:HL was therefore very different between the two groups (1:1.8 in the project population, 3.1:1 in the HMDS population).

Regarding T-cell LPDs the numbers in the project population are small but the ratio of T:B cell LPDs was unusual. In the project population there were more cases of T-ALL than B-ALL (4.9% vs 3.3%) whereas the reverse is usually seen (0.4% vs 1% in the HMDS population). There were also almost as many cases of PTCL as DLBL in the project population (4.9% vs 6.6%) whereas PTCL is considerably less common than DLBL in the UK (1.2% vs 21.8% in the HMDS population). Comparing the diagnoses in the 2 groups there was a statistically significant difference for T-ALL (**p<0.0001**) and for PTCL (**p=0.0153**), but not for B-ALL (p=0.0959).

We had expected to see a higher proportion of BL amongst the project LPD patients than the 3.3% seen. This is similar to the proportion of cases seen at HMDS (2.2% of LPDs, p=0.5895).

In the project there were no cases of HCL or extranodal MZL. This is probably due to a combination of the rarity of these conditions and the small numbers in the project, as well as the very indolent nature of these conditions. Extranodal MZL is commonly diagnosed on upper gastrointestinal endoscopy so the lack of a routine endoscopy service in Ghana, as discussed in Chapter 1, probably also contributed.

SMZL

Clinical features

In the 18 patients with SMZL, there was a 1:3.5 M:F ratio. The median age was 64.5 years (range 40-78 years). Abdominal symptoms (pain, distension or the sensation of an

abdominal mass) were the presenting complaint in 14 patients (78%). Only 1 patient presented with symptomatic lymphadenopathy. 3 patients presented with other non-specific symptoms. On direct questioning 17 patients (94%) reported B symptoms. 17 patients (94%) were performance status 1 and 1 patient was performance status 3.

All the patients with SMZL had palpable splenomegaly at presentation with a median of 17cm palpable (range 6-30cm). 15 patients (83%) had palpable hepatomegaly in addition to their splenomegaly with a median of 5cm palpable (range 2-17cm). 3 patients (17%) had palpable lymphadenopathy >1cm in diameter. 16/18 patients (89%) had a lymphocytosis, 6/7 (86%) had an elevated LDH but none of the 8 patients tested for HIV were found to be positive. Unfortunately it was not possible to perform serum protein electropheresis or measure immunoglobulin or beta-2 microglobulin levels locally. The other blood results at presentation are shown in table 4.1.

Table 4.1 – SMZL patients' blood results at presentation

Haemoglobin (g/dl) median (range)	8.5 (3.6-13.5)
WCC ($x10^9$/l) median (range)	53.7 (2.8-UR)
Platelets($x10^9$/l) median (range)	114 (14-181)
Lymphocytes ($x10^9$/l) median (range)	30.1 (1.3-121.3)
Creatinine (μmol/l) median (range)	97.2 (35-141)
LDH (U/l) median (range)	424 (167-585)

Normal ranges: Haemoglobin 11.1-18 g/dl, WCC 2.5-10 $x10^9$/l, Platelets 140-440 $x10^9$/l, Lymphocytes 0.6-4.1 $x10^9$/l, Creatinine 53-124 μmol/l, LDH 100-190 U/l.
UR=unrecordable.

Pathological features

All the SMZL patients had marrow involvement at diagnosis. The lymphocytes were small, round and had a low rate of proliferation (median Ki-67 positive fraction 5%). Cytoplasmic projections were noted in a minority of patients. Marrow infiltration was diffuse with a loss of fat spaces in 7 (39%), interstitial with preservation of fat spaces in 3 (17%), nodular in 4 (22%), both interstitial and nodular in 3 (17%) and both interstitial

and intrasinusoidal in1 (6%). No patients with an exclusively intrasinusoidal pattern of infiltration were noted.

Phenotypic features

All cases were assessed by standard streptavidin-biotin immunohistochemistry using the following panel of antibodies – CD3, CD5, CD10, CD20, CD23, CD79, Cyclin D1, BCL2, BCL6, MUM1, Ki-67. All cases expressed the pan B cell antigens CD20 and CD79 as well as BCL2, but were typically negative with CD5, CD10, CD23, Cyclin D1, BCL6 and MUM1.

Genotypic features

Cases were initially screened for *IGH* rearrangement using an *IGH* breakapart probe set and for deletions of 6q21 and 7q31. Normal patterns were obtained in 12 of the 18 cases assessed. In 6 cases the *IGH* breakapart probe set gave an abnormal pattern. This comprised an unbalanced rearrangement in 2 cases, low level rearrangement in 3 cases and apparent monosomy 14 in 1 case. These cases were further investigated with *CYCLIN D1/CCND1, PAX-5* and *MALT1* breakapart probe sets although no rearrangements were seen. 7q deletions were demonstrated in 4 patients (22%) whilst 1 patient had a 6q deletion and a further patient had additional copies of the probe suspicious of trisomy 6.

IGHV sequencing

IGHV sequencing was performed in 15 cases – 9 were considered to be unmutated and 6 mutated using the conventional cut-off of 2%. The divergence and junctional analysis results are shown in table 4.3. The majority of cases utilised VH3 family genes. Within the mutated group the overall mutation load was relatively low – median 3.2% (range 3-4.2%).

Table 4.3 – SMZL *IGHV* sequencing results

Unmutated cases Divergence (homology)	Mutated cases Divergence (homology)
3/248bp from VH4-31 (98.8%)	9/241bp from VH4-39 (96.3%)
1/269bp from VH3-11 (99%)	7/227bp from VH3-30 (96.9%)
3/243bp from VH3-33 (98.7%)	8/217bp from VH2-70 (97%)
0/245bp from VH4-39 (100%)	9/214bp from VH3-11 (95.8%)
0/242bp from VH3-23 (100%)	8/246bp from VH3-21 (96.7%)
1/243bp divergence from VH3-7 (99%)	7/241bp from VH3-48 (97%)
2/240bp from VH3-7 (99%)	
2/246bp from VH3-21 (99.2%)	
2/247bp from VH3-23 (99%)	

Discussion of results in patients with SMZL

The term SMZL, first used by Schmid *et al* in 1992, was a provisional entity in the REAL classification and later a distinct clinicopathological entity in the WHO classification (Schmid *et al*, 1992; Harris *et al*, 1994; Jaffe *et al*, 2001). SMZL, as classified by the WHO, is a B-cell neoplasm comprising small lymphocytes which surround and replace the splenic white pulp germinal centres, efface the follicle mantle and merge with a peripheral (marginal) zone of larger cells including scattered transformed blasts, with both small and larger cells infiltrating the red pulp (Jaffe *et al*, 2001).

Matutes *et al* (2008) recently published proposals for a revision of SMZL diagnostic, staging and therapeutic criteria. (Figure 4.1) Although intrasinusoidal marrow infiltration is included as one of the diagnostic criteria, the authors note that in advanced cases there is a combination of intrasinusoidal and nodular infiltration and that in a minority of cases interstitial infiltration also occurs. Spleen histology was not available in our patients as splenic biopsy was not available locally therefore the patients were diagnosed on clinical features and bone marrow findings alone. As the patients had such advanced disease most (94%) no longer had an intrasinusoidal pattern of infiltration on their trephines although they had a typical blood and marrow morphology and immunophenotype.

Figure 4.1 – Minimum diagnostic criteria for SMZL (Matutes *et al*, 2008)

Either:

1) spleen histology + immunophenotype with a 'CLL score' of 2 or less, or
2) typical blood and bone marrow morphology + immunophenotype + intrasinusoidal infiltration by CD20-positive cells (if spleen histology is unavailable).

In developed countries SMZL patients have a median age at diagnosis of 65 years and an equal male to female ratio (Oscier *et* al, 2005; Matutes *et al*, 2008). The median age in our cohort of Ghanaian SMZL patients was 64.5 years. There was a striking difference to the published data in the male to female ratio which was 1:3.5 in our cohort. This female preponderance appears to be confined to younger patients (see table 4.4 – patients split into Group A - patients less than 58 years and Group B – patients 58 years and older).

Table 4.4 – Sex ratio and age of SMZL patient subgroups

	M:F	Median age (range)
Group A	0:7	48 years (40-57 years)
Group B	4:7	75 years (58-78 years)

It is therefore possible that the cause of the excess cases of SMZL in this population might be something to which young women are particularly susceptible, for example malaria. Malaria is endemic in Ghana and there is an altered immunological response to malaria during pregnancy (Rogerson *et al*, 2007). This altered immunological response might account for the increased number of cases of SMZL we found in women compared to men. As discussed in chapter 1, an infectious aetiology has been postulated in other subtypes of MZL and so it is possible that an infection accounts for the excess cases of SMZL seen in this project and perhaps also accounts for the atypical sex ratio seen.

The role of antigenic stimulation is well established in MALT lymphoma (see Chapter1). In SMZL antigenic stimulation due to HCV infection has been implicated in some studies

(Hermine *et al*, 2002). In this context a possible relationship between chronic malaria infection, HMS and lymphoma has been suggested (Bates *et al*, 1992; Bates & Bedu-Addo, 1997a; Wallace *et al*, 1998; Bates *et al*, 2001). There are, however, many possible causes of splenomegaly in a tropical setting such as schistosomiasis, leishmaniasis, haemoglobinopathies as well as other haematological disorders. It is therefore not possible to diagnose SMZL simply on the basis of splenomegaly that has not responded to proguanil.

In developed countries SMZL may be an asymptomatic finding – for example the detection of mild splenomegaly or asymptomatic lymphocytosis on routine medical screening, or it may present with symptoms – frequently those of symptomatic splenomegaly, commonly with associated B symptoms (Thieblemont *et al*, 2003). All of the patients in our group were symptomatic at presentation and most presented with both abdominal symptoms and B symptoms. Very little routine health screening occurs in Ghana and therefore patients do not tend to be diagnosed with asymptomatic disease.

1 patient in our group presented with symptomatic lymphadenopathy and 2 others had 1 or more palpable nodes ≥2cm in diameter. There has been some discussion as to whether patients with enlarged nodes, at sites other than the splenic hilum, should be classified as SMZL as opposed to nodal MZL. The WHO classification suggests that patients with SMZL do not have enlarged nodes other than those at the splenic hilum – some authors do not agree, however and include patients with enlarged nodes in the SMZL diagnostic category if they have a predominately splenic presentation along with the other features of SMZL (Jaffe *et* al, 2001; Thieblemont *et al*, 2003; Chacón *et al*, 2002).

The patient that presented with symptomatic lymphadenopathy had extensive disease at presentation - 19 cm palpable, but asymptomatic splenomegaly and extensive intra-abdominal nodes on ultrasound scan – it is likely that he had had asymptomatic SMZL for some time, but that it had progressed over time to include nodal areas. In the 2 patients with clinically significant, but localised lymphadenopathy, splenic enlargement was the predominant feature of their disease - their palpable spleen sizes were 17 and 23

cm and they presented with abdominal pain. It is therefore appropriate to classify these 3 patients as having SMZL as opposed to nodal MZL.

89% of the patients were anaemic. In European literature, cytopenias occurring in patients with SMZL typically reflect hypersplenism, but in our cohort marrow infiltration was also a factor (Matutes *et al*, 2008). Other factors such as haematinic and other vitamin deficiencies, thalassaemias and infections probably also contributed, although there was no obvious haemolysis. There was a wide range in WCC – from low due to hypersplenism, to high due to late presentation with heavy marrow involvement and peripheral blood overspill. There is discussion in the literature regarding whether patients with peripheral blood involvement should be classified as SLVL rather than SMZL (Thieblemont *et al*, 2002). The WHO classification however does not have a separate SLVL category and includes patients with peripheral blood involvement in the SMZL category (Jaffe *et al*, 2001).

As LDH results were only available in 7 of our SMZL patients and albumin in 4, it was not possible to accurately score the patients according to the SMZL prognostic scoring described by Arcaini *et al* (2006). This scoring system places patients into low (no adverse factors), intermediate (1 adverse factor) and high risk (2 or 3 adverse factors) groups based on haemoglobin, LDH and albumin results with a 5 year cause-specific survival of 88%, 73% and 50% respectively. Using the results that were available, however, most of the patients were probably high risk as even with the limited results only 1 patient was possibly low risk (pending both LDH and albumin results), 11 were at least intermediate risk (8 pending both albumin and LDH results) and at least 6 were high risk.

SMZL does not have a disease defining immunophenotype and diagnoses are through exclusion with SMZL typically lacking markers seen in CLL (CD5 and CD23) and FL (CD10 and BCL6) (Isaacson *et al,* 1994; Matutes *et al,* 1994; Troussard *et al,* 1996; Oscier *et al*, 2005). Our cases were positive for CD19, CD20, CD22, CD79b, FMC7, Bcl-

2 and negative for CD5, CD10, CD23, BCL6 and CYCLIN D1 with a low rate of proliferation.

SMZL does not have a specific underlying genotypic abnormality; however 83% of cases have chromosome gains or losses by comparative genomic hybridization with a median of 4 abnormalities per case (Matutes *et al*, 2008). The most common cytogenetic change detected by FISH is chromosome 7q deletion (30-40% of cases – commonly loss of 7q32) (Matutes *et al*, 2008). Numerous other cytogenetic abnormalities have been reported particularly gain of 3q and 12q, but translocations involving Ig heavy and light loci are uncommon (Andersen *et al*, 2004; Matutes *et al*, 2008).

Conventional cytogenetic analysis was not possible in our patients. Interphase FISH was therefore performed on blood and marrow smears. In our cohort of patients we demonstrated del 7q in 22%. Analysis of *IGH* in our series demonstrated an apparent unbalanced rearrangement in 2 patients and low level rearrangement in a further 3 patients. The significance of these findings is unclear. There was, on further assessment, no evidence of *CCND1*, *BCL2* or *PAX5* rearrangements in these cases. Similarly there was no evidence of *MALT1* rearrangements using a dual colour breakapart assay.

When *IGHV* sequence analysis was first performed on 5 cases of SLVL, all 5 cases showed somatic mutation from germline sequences, but no clonal heterogeneity was detected (Zhu *et* al, 1995). Subsequent work on a further 156 SMZL cases has however shown that approximately two thirds of the cases are mutated and one third are unmutated as shown in table 4.5. We found a slightly higher proportion of unmutated cases (9/15, 60%). This may reflect late presentation and possibly poor prognostic disease in our cases. Ruiz-Ballesteros *et al* (2005) in a series of post-splenectomy SMZL patients found that unmutated cases had a worse prognosis than mutated cases, similar to that found in CLL; however other authors have not confirmed these findings.

Table 4.5 – Summary of published *IGHV* sequence analysis results in SMZL

Reference	Number of cases	Mutated: Unmutated	Comments
Zhu *et al*, 1995	5	5:0	SLVL cases
Tierens *et al*, 1998	4	4:0	Compared SMZL cases with other MZL subtypes
Dunn-Walters *et al*,1998	4	4:0	
Miranda *et al*, 1999	4	3:1	Preferential use of VH_1 family genes noted
Bahler *et al*, 2002	8	4:4	
Zhu *et al*, 2002	4	1:3	Evidence of initiation of somatic mutation in vivo
Algara *et al*, 2002	35	18:17	18/40 sequences used VH_{1-2} segment
Tierens *et al*, 2003	23	16:7	11 mutated cases showed evidence of Ag selection
Traverse-Glehen *et al*, 2005	35	24:11	VH_1 used in 13, Ag driven in 8
Papadaki *et al*, 2007	34	21:13	VH_4 family in 15 cases, VH_3 family in 9 cases and VH_{1-2} in 3 cases
Total	**156**	**100:56**	

In our group of patients VH3 predominates in line with the distribution in normal cells (Brezinschek *et al*, 1997). Preferential use of VH1-2, as reported in some series, was not seen (Miranda *et al*, 1999; Algara *et al*, 2002; Traverse-Glehen *et al*, 2005).

The mutation load was relatively low in our cases (median 3.2%, range 3-4.2%) compared to the published literature - in the 45 mutated cases reported by Papadaki *et al* (2007) and Traverse-Glehen *et al* (2005), the median mutation load was 7% (range 2.1-13.5%).

IGHV sequence analysis is a powerful investigative tool which can provide insight into the pathogenesis of B-cell LPDs and in particular the role of antigens or superantigens in driving neoplastic transformation (Ghia *et al*, 2009). The presence of *IGHV* mutations per se is however not evidence of antigenic drive but merely demonstrates that the clonal cells have at some stage encountered antigen and undergone the process of somatic hypermutation. A restricted repertoire of *IGHV* genes used by individual tumours when compared to the normal B cell repertoire supports the role of antigen in pathogenesis and an example of this is the preferential use of VH1-69 in CLL, salivary MZL and HCV associated LPDs.

This concept has been extended with the concept of so-called 'stereotyped receptors'. By screening a large number of CLL cases it has been possible to demonstrate specific groups of cases with highly homologous B-cell receptors. This was first demonstrated in CLL when a significant proportion of patients utilizing the IGHV3-21 gene were found to have highly homologous CDR3 regions and restricted use of IGHJ6 and IGLV3-21 joining and light chain genes respectively (Ghia *et al*, 2009).

Further analysis involving almost 2000 patients has identified evidence of restricted or stereotyped receptors in 26% of CLL cases (Ghia *et al*, 2009). Other examples include the *IGHV*1-69, *IGHD*3-16, *IGHJ*3 and *IGKV*3-20 combination and the *IGHV*4-39, *IGHD*6-13, *IGHJ*5 and *IGK*1-39 / *IGKV*1D-39 (Ghia *et al*, 2009). Interestingly the combinations seem correlate to some extent with the clinical phenotype (Ghia *et al*, 2009). For example the latter combination is predominately seen in IgG positive CLL cases in which there is a female preponderance and aggressive clinical course. This is an interesting observation given the sex bias seen in our cases of SMZL. A further interesting observation is that the *IGHV*3-21 stereotype appears to be restricted to northern Europe which further supports the role of specific antigen in these cases (Ghia *et al*, 2009).

Criteria for defining stereotyped receptors have been developed and these suggest that they are defined by the use of the same *IGHV/D/J* germline genes, usage of the same

IGHD reading frame and VH CDR3 amino acid identity ≥60% (Ghia *et al*, 2009). It is has also become clear that the CDR3 amino acid sequence homology can also be achieved using different but phylogenetically related germline *IGHV* genes. We plan in the first instance to sequence the immunoglobulin light chain genes in our cases although large numbers of cases are likely to be required for a definitive assessment.

CLL

Clinical features

In the 12 patients with CLL the median age was 60.5 years (range 45-80 years) and the M:F ratio was 1:2. The presenting complaint was symptomatic lymphadenopathy in 5 patients (42%), abdominal symptoms in 3 (25%) and non specific symptoms in 4 (33%). On direct questioning 11 patients (92%) reported B symptoms. 11 patients (92%) were performance status 1 and 1 patient was performance status 2.

8 of the CLL patients (67%) had palpable splenomegaly with a median of 8.5cm palpable (range 4-22cm), 6 (50%) had palpable hepatomegaly with a median of 3.5cm palpable (range 3-7cm) and 7 (58%) had significant palpable lymphadenopathy. Most of the patients were anaemic and had a lymphocytosis (8/12 and 10/12 respectively). The 2 patients with low level marrow infiltration did not have a lymphocytosis however and had predominately nodal disease. The LDH was elevated in all 8 patients that were tested, but none of the 3 patients tested for HIV were found to be positive. Unfortunately it was not possible to perform serum protein electropheresis or measure immunoglobulin or beta 2 microglobulin levels locally. The other blood results and Binet staging at presentation are shown in table 4.6.

Table 4.6 – CLL patients' blood results and Binet Stage at presentation

Haemoglobin (g/dl) median (range)	9.8 (2.1-15)
WCC ($x10^9$/l) median (range)	66.8 (4.1-265)
Platelets($x10^9$/l) median (range)	145 (37-244)
Lymphocytes ($x10^9$/l) median (range)	55.4 (1.7-201.8)
Creatinine (μmol/l) median (range)	106 (62-132)
LDH (U/l) median (range)	326 (221-1192)
Binet Stage A	2/12 (17%)
Binet Stage B	2/12 (17%)
Binet Stage C	8/12 (66%)

Normal ranges: Haemoglobin 11.1-18 g/dl, WCC 2.5-10 $x10^9$/l, Platelets 140-440 $x10^9$/l, Lymphocytes 0.6-4.1 $x10^9$/l, Creatinine 53-124 μmol/l, LDH 100-190 U/l.

Pathological features

1 patient was diagnosed on PB flow cytometry and did not have a bone marrow as she was elderly, frail and had a high WCC. In 9 patients there was extensive primarily diffuse infiltration while low level interstitial infiltration was noted in the remaining 2 patients.

2 patients had lymph node biopsies and these demonstrated the typical morphological features of CLL as they both contained ill-defined proliferation centres or pseudofollicles containing larger nucleolated cells. The rate of cell proliferation was low in all cases (Ki-67 typically <10%).

Phenotypic features

2 cases were assessed by PB or BMA flow cytometry, 9 by immunohistochemistry on the trephine and both modalities were performed in 1 case. In addition, immunohistochemistry was performed on lymph node sections in 2 cases. The majority of cases were characterised by the standard immunophenotypic features seen in CLL although one case had weak CD5 expression while another lacked expression of CD23. All cases lacked CYCLIN D1 protein.

Genotypic features

FISH studies were not performed. We considered that the small numbers of cases available precluded a meaningful assessment of the relative frequency of the known cytogenetic prognostic factors namely deletions of 11q, 13q and 17p.

IGHV sequencing

IGHV sequencing was performed in 10 cases – 7 were considered unmutated and 3 mutated using the conventional 2% cut-off. The divergence and junctional analysis results are shown in table 4.7.

Table 4.7 – CLL *IGHV* sequencing results

Unmutated cases **Divergence (homology)**	**Mutated cases** **Divergence (homology)**
0/253bp from VH6-1 (100%)	17/243bp from VH4-61 (93%)
1/236bp from VH1-69 (99%)	17/248bp from VH3-43 (93%)
2/237bp from VH1-69 (99.2%)	10/238bp from VH4-39 (95.8%)
3/236bp from VH1-69 (98.7%)	
1/236bp from VH1-69 (99%)	
1/250bp from VH1-2 (99%)	
0/244bp from VH4-34 (100%)	

Discussion of results in patients with CLL

Although the numbers are small, with only 12 patients diagnosed with CLL, the sex ratio is the reverse of that seen in CLL in the West as there were twice as many female patients as male (Oscier *et al*, 2004). A similar result was found in Benin City, Nigeria where the M:F ratio of 60 patients diagnosed with CLL between 1995 and 2005 was 1:3 (Omoti *et al,* 2007). As the Nigerian patients were diagnosed on morphology alone, and as malaria is epidemic in Nigeria, it is possible that this group may have included some patients with SMZL as opposed to CLL and as discussed earlier this may contribute to the excess

numbers of female patients. The median age was similar to that usually found in CLL (Oscier *et al*, 2004).

As discussed earlier all the patients were symptomatic at diagnosis as routine blood tests are rarely done in Ghana so asymptomatic disease is rarely, if ever, diagnosed. This is in contrast to the west where 70-80% of CLL patients are diagnosed as an incidental finding on a routine FBC (Oscier *et al*, 2004). Two thirds of the Ghanaian CLL patients were Binet stage C compared to one tenth of UK CLL patients probably reflecting late presentation (Oscier *et al*, 2004). It would therefore be very difficult to compare the epidemiology of CLL in Ghana with that in the West by simply comparing the number of diagnoses made. One way to see whether there is a true difference in the incidence of CLL in Ghana compared to the published literature would be to look for occult disease in asymptomatic patients. Approximately 4% of the population of the UK have occult CLL by flow cytometry (Rawstron *et al*, 2002). A similar study in Ghana would be very informative and would be the only way to see if there was a real difference in the incidence of CLL between the two countries. This has been done to some extent with MGUS as discussed in Chapter 1 (Landgren *et al*, 2006).

There was a higher incidence of VH1-69 in our cohort than expected (Hamblin *et al*, 1999). This is a high risk feature in CLL (Guarini *et al*, 2003). An antigen driven process can be implied from the presence of stereotyped CDRIII regions, therefore sequencing a large number of cases is therefore likely to be very informative (Stamatopoulos *et al*, 2007).

Aggressive Lymphomas

Clinical features

16 patients were diagnosed with histologically aggressive lymphoma – 7 with HL, 4 with DLBL, 3 with PTCL and 2 with BL. The demographics, presenting symptoms and signs, blood results, stage and prognostic index for these patients are shown in tables 4.8 and 4.9.

Table 4.8 – Clinical features of patients with aggressive lymphomas

	HL N=7	DLBL N=4	PTCL N=3	BL N=2
Age years median(range)	18 (14-69)	55 (37-79)	30 (12-69)	33 (13-50)
M:F	1:1.3	1:1	3:0	1:1
Abdominal symptoms	3 (42%)	0 (0%)	0 (0%)	0 (0%)
Symptomatic nodes/masses	2 (29%)	4 (100%)	2 (66%)	1 (50%)
Non-specific presentation	2 (29%)	0 (0%)	1 (33%)	1 (50%)
B symptoms present	7 (100%)	3 (75%)	2 (66%)	2 (100%)
Performance status 0-1	3 (43%)	3 (75%)	2 (66%)	1 (50%)
Performance status 2-3	2 (29%)	1 (25%)	1 (33%)	1 (50%)
Performance status 4	2 (29%)	0 (0%)	0 (0%)	0 (0%)
Palpable splenomegaly	5 (71%)	1 (25%)	0 (0%)	0 (0%)
Palpable hepatomegaly	4 (57%)	1 (25%)	1 (33%)	0 (0%)
Palpable nodes >1cm diameter	4 (57%)	4 (100%)	3 (100%)	0 (0%)
Haemoglobin (g/dl) median (range)	5.8 (4.7-6.8)	10.3 (5-10.4)	13.3 (11.1-14.7)	9.1 (6.1-12.2)
WCC ($x10^9$/l) median (range)	3.2 (1.4-21.9)	4.7 (3.9-8.6)	6.9 (5.9-15.3)	7.7 (3.1-12.3)
Platelets($x10^9$/l) median (range)	39 (6-518)	158 (31-433)	289 (211-295)	229 (200-258)
Creatinine (µmol/l) median (range)	74 (53-88.4)	133 (80-185)	88.4	110 (71-150)
LDH (U/l) median (range)	606 (372-840)	315 (226-405)	764 (384-1144)	622
LDH elevated/number tested	2/2	3/3	2/2	1/1
Positive HIV test/number tested	0/7 (0%)	0/4 (0%)	0/1 (0%)	1/1
BM involvement at diagnosis	6 (86%)	2 (50%)	1 (33%)	1 (50%)

Normal ranges: Haemoglobin 11.1-18 g/dl, WCC 2.5-10 $x10^9$/l, Platelets 140-440 $x10^9$/l, Creatinine 53-124 µmol/l, LDH 100-190 U/l.

Table 4.9 –Stage and IPI of patients with aggressive lymphomas

	HL N=7	DLBL N=4	PTCL N=3	BL N=2
Ann Arbor Stage	2B - 14% 4B - 86%	1B – 25% 2A – 25% 4B – 50%	3B – 33% 4A – 33% 4B – 33%	4B – 100%
IPI (where LDH available)	Not applicable	1HI, 1LI, 1 low	2 HI	1 HI

HI=high intermediate, LI=low intermediate

Pathological and immunophenotypic features

Diagnosis of HL was confirmed using the following immunohistochemistry panel: CD3, CD20, CD15, CD30, MUM1, OCT2, BOB1. Cases were typically CD30 positive, CD20 negative and MUM1 positive with variable expression of CD15 and the immunoglobulin transcription factors OCT2 and BOB1. Of the 7 patients with HL all had classical HL - 5 were NS and 2 were MC. The diagnosis in 5 patients was made on bone marrow trephine. 1 of these had also had a lymph node biopsy reported locally, but unfortunately we were unable to obtain the tissue block. This patient is discussed in detail in Chapter 5, case 1.

1 patient with NSHL was diagnosed on both node biopsy and bone marrow trephine, another was diagnosed on a lymph node biopsy and did not have marrow involvement. This patient had had a lymph node biopsy reported locally as showing granulomatous lymphadenitis and been commenced on anti-tuberculosis treatment, but had continued to deteriorate clinically so was referred back to the haematology clinic and a repeat biopsy was arranged. Again we were unable to obtain the tissue block from the first biospy.

3 of the 4 DLBL diagnoses were made on lymph node biopsies. Of these, 1 patient also had marrow involvement, 1 did not and in the third the trephine was not adequate for staging. The 4th patient had had a lymph node biopsy reported locally as showing NHL ("immunoblastic sarcoma") and had marrow involvement on their trephine. All cases were characterized by the following immunohistochemistry panel: CD3, CD5, CD10, CD20, CD23, CD79, BCL2, BCL6, MUM1, FOXP1, Ki-67. Only 1 case had a germinal

centre immunophenotype as per the criteria published by Hans *et al* (2004). The other 3 cases had a non-germinal centre immunophenotype indicative of poor overall survival (Hans *et al*, 2004). All cases expressed FOXP1 which is associated with adverse outcome in DLBL (Barrans *et al*, 2004).

The PTCL cases were characterised using the following immunohistochemistry panel: CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD30 and Ki-67. Of the 3 cases of PTCL, 1 had had a lymph node biopsy reported locally as showing high grade NHL – immunohistochemistry and review of the tissue block at HMDS demonstrated PTCL. This patient did not have marrow involvement. The lymph node biopsy in the 2nd patient was necrotic, but PTCL was diagnosed on the trephine. In the 3rd patient the diagnosis was made on lymph node biopsy and the trephine was not adequate for staging.

One of the patients with BL was diagnosed on bone marrow trephine and the other was diagnosed on breast mass biopsy and did not have marrow involvement. Both cases were characterised by typical Burkitt morphology and immunophenotype: CD10 positive, CD20 positive, BCL2 negative, BCL6 positive, p53 positive, p21 negative, Ki-67 100%. The t(8;14) was demonstrated in the patient with the breast masses. It was not possible to demonstrate t(8;14) in the other BL patient as the only tissue available was resin embedded bone marrow trephine which is unsuitable for FISH.

Discussion of results in patients with aggressive lymphomas

86% of the patients with HL and 50% of those with DLBL had marrow involvement at presentation. This is very different to that seen in the West where marrow involvement occurs in ~5% of patients with HL and ~27% of patients with DLBL (Howell *et al*, 2002; Campbell *et al*, 2006). This is probably due to patients presenting late in the course of their disease. Although the numbers are small the presenting symptoms in the patients with HL were also atypical - only 2/7 (29%) HL patients presented with symptomatic lymphadenopathy; the rest presented with abdominal or non-specific symptoms. In the West, 75% of cases of Classical HL present with cervical lymphadenopathy (Jaffe *et al*, 2001).

In this cohort of patients there was an apparent relative excess of HL and PTCL over DLBL when compared to the HMDS data. This may be due to chance given the small numbers, but is certainly worthy of further study.

Another interesting observation in this study was the relatively low incidence of BL. A minimum age of 12 years was deliberately chosen in order to exclude patients with classical endemic BL. Of the two patients in this cohort one was aged 13 years and had the typical features of advanced endemic BL with multiple abdominal masses and pancytopenia and the other was HIV positive and presented with bilateral breast masses. It is interesting to note that prolactin receptors have been reported on BL cells and so bilateral breast involvement is not an uncommon presentation of BL during pregnancy and puberty, although neither was the case in this patient (Jaffe *et al,* 2001).

Other LPDs

Myeloma

6 patients had myeloma with a median age of 61 years (range 50-74 years) and an equal M:F ratio. 2 patients were performance status 1 and 4 were performance status 3-4. 1 patient had palpable hepatosplenomegaly, the rest had no organomegaly or lymphadenopathy. All the patients were anaemic with a median Haemoglobin 7.65 g/dl (range 5.1-8.4g/dl). Serum creatinine was elevated in 2/5 patients tested and calcium was elevated in 2/4. Lytic lesions were present in 3 patients (50%). There was extensive marrow infiltration by plasma cells in all and diagnoses were confirmed by demonstrating cytoplasmic light chain restriction and/or aberrant plasma cell phenotype in all cases. We were not able to calculate the International Staging System as we were unable to measure beta-2 microglobulin (Greipp *et al*, 2005). We were also unable to do immunoglobulin quantification as well as serum and urine electropheresis. FISH studies were not performed as there were too few cases to make a meaningful assessment of known cytogenetic abnormalities, particularly deletion of chromosome 13 and t(4;14).

ALL

5 patients had ALL – 3 T-lineage and 2 B-lineage. In all cases there was extensive marrow infiltration by lymphoid blast cells with B- or T- precursor immunophenotypes and expression of tdt. The median age was 25 years (range 12-62 years) and the M:F ratio was 3:2. 2 patients (40%) had palpable splenomegaly and 4 (80%) had significant lymphadenopathy. The median haemoglobin was 7.1g/dl (range 3.4-11.4 g/dl), median WCC $30x10^9$/l (range 3-$100x10^9$/l) and median platelet count $21x10^9$/l (range 13-$158x10^9$/l).

FL, MCL and NK cell LPD

2 patients had FL, 1 had MCL and 1 had NK cell LPD. The patient with NK cell LPD is presented in Chapter 5 (case 5). Significant splenomegaly was noted in the patient with MCL and one of the patients with FL confirming the value of formal haematopathological assessment in patients with splenomegaly.

Discussion of results in patients with other LPDs

The number of patients in this cohort is too small to make any meaningful assessment about the relative incidence rates of myeloma, ALL and other LPD's. However it is interesting to note that a relative excess of T-lineage ALL has previously been reported in some studies from Egypt and Morocco (Kamel *et al*, 1989; Hussein *et al*, 2004; Dakka *et al,* 2007).

Summary

- This study has demonstrated that it is possible to perform complex immunophenotypic, cytogenetic and molecular analysis in West Africa
- This small cohort of patients has provided an insight into the spectrum of LPDs seen in Ghana
- Further study is clearly worthwhile and utilising the model we have developed it should be possible to make a definitive assessment of LPDs in Africa

Chapter 5 – Interesting cases

Introduction

In this chapter 5 interesting cases are presented and discussed as each highlights different aspects of the impact of the project on patient management.

Case 1

Case summary

18 year old patient with stage 4B HL. This case illustrates the common problem of late presentation and the need for pragmatism in clinical decision making. The project enabled the diagnosis to be confirmed and informed the decision regarding treatment duration.

Presentation

An 18 yr old, single, female, student presented with her parents (a midwife and a businessman) to medical outpatients at KATH on 23rd November 2004 with fevers, sweats, weight loss, fatigue and a 3 cm left submandibular node. She had a history of a transient occipital node when she was 5 years old followed by a persistent left supraclavicular node when she was 12½. This was removed surgically, but was not sent for histology as the family could not afford it at the time. A further left supraclavicular node was removed when she was 14 years old, but not sent for histology as the family again could not afford it.

The patient was referred from medical outpatients to the surgeons for a further biopsy which was performed on 1st December 2004 and reported by a private laboratory on 12th February 2005 as showing NS HL. She was referred to the haematology outpatient department and entered into the LPD study on 14th March 2005. On examination she had

3 pea-sized left supraclavicular nodes and 3 biopsy scars. No disease was palpable elsewhere.

Investigation results

On entry to the project the following results were available locally: haemoglobin 6.5 g/dl, WCC 5.7 x10^9/l, platelets 255 x10^9/l, MCV 73.1 fl, creatinine 53 µmol/l, ALT 41U/l, HIV test negative. No radiological investigations were performed. The lymph node biopsy had been reported as above and we requested the block from the pathologist without success. Bone marrow aspirate and trephine were performed and reported at HMDS. The trephine showed NS HL with near maximal cellularity. Normal haematopoesis was replaced by dense fibrosis with a background population of reactive T cells and histiocytes. Scattered throughout were large numbers mononuclear Hodgkin cells and classical bi-nucleate Reed Sternberg cells. (Figures 5.1-5.3)

Figure 5.1 – Case 1 Hodgkin Lymphoma – H&E section x20 magnification

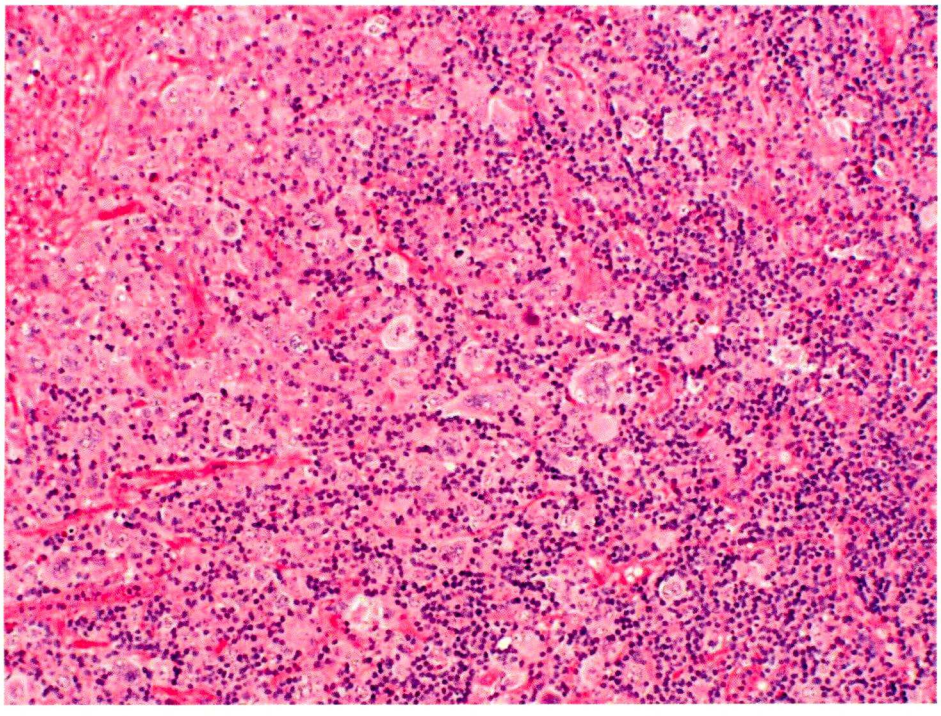


Figure 5.2 – Case 1 Hodgkin Lymphoma – H&E section x60 magnification

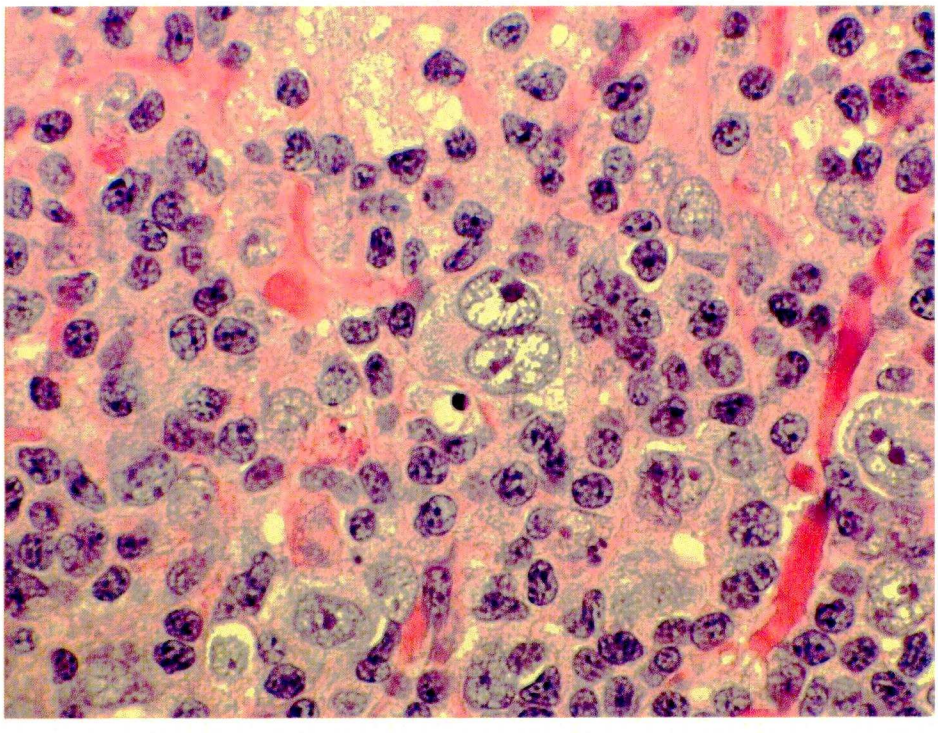
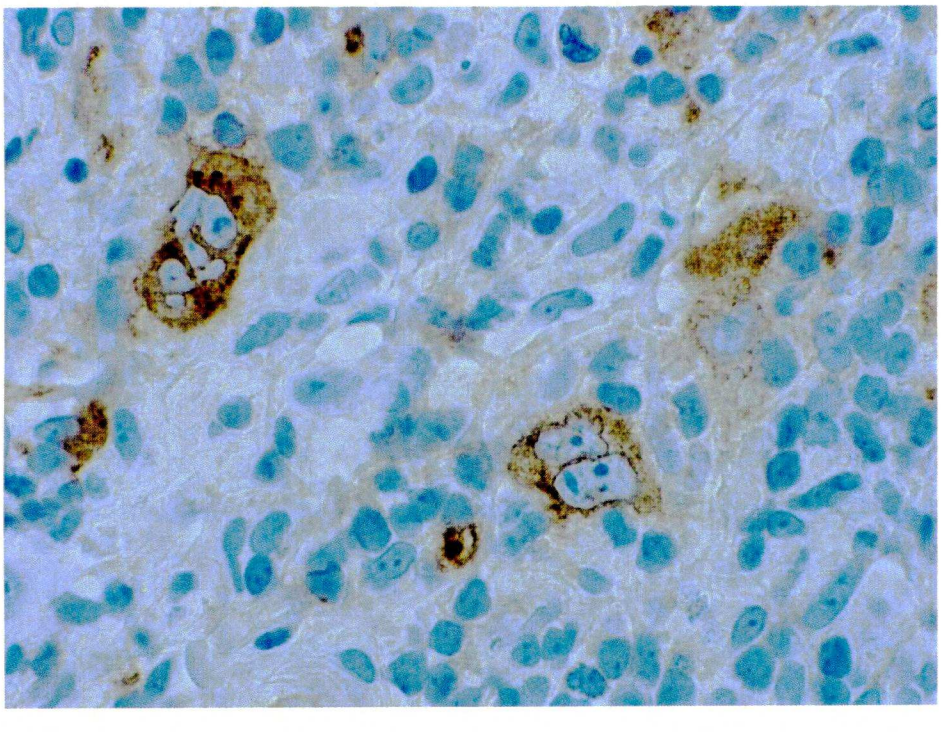


Figure 5.3 – Case 1 Hodgkin Lymphoma – CD30 Immunohistochemistry



Treatment

She was transfused and commenced combination chemotherapy with cyclophosphamide, doxorubicin, vincristine, prednisolone (CHOP). After 1 cycle of chemotherapy the previous pea-sized nodes had fully resolved and after 4 cycles a repeat bone marrow trephine showed no evidence of infiltration. She received a total of 6 cycles of CHOP chemotherapy, which, although expensive, her parents were just able to afford. At 6 month follow-up she was well and her blood count was normal other than a persisting microcytosis (likely alpha thalassaemia trait). At 12 month follow-up she remained well with no evidence of disease recurrence and a stable blood count.

Discussion

This case raises some issues for discussion. The first is that of late presentation of disease either due to a delay in seeking medical attention or, as in this case, due to an inability to afford investigation or treatment. It is likely that her long term prognosis would have been better if she had been treated earlier, when her disease would have been less extensive, possibly even localised.

Regarding the other cases of HL in the study the late presentation also affected the outcome. 4 out of the 7 cases of HL presented with pancytopenia and hepatosplenomegaly, but without significant palpable lymphadenopathy and the diagnosis was made on bone marrow trephine alone. These patients had a particularly poor outcome – 1 was an outpatient and did not attend for any follow-up after her bone marrow biopsy. The other 3 were inpatients and died shortly (median 5 days) after entry into the study despite supportive care including corticosteroids. If the diagnosis had been made earlier in the disease process and chemotherapy started their outcome would probably have been better.

As mentioned in chapter 4, 6 out of the 7 (86%) cases of HL in this project had marrow involvement at diagnosis. This probably reflects late disease presentation. In the UK,

marrow involvement at diagnosis is seen in 5% of classical HL cases (Howell *et al*, 2002).

The second issue is that of the cost of tests for diagnosis and staging. In this case 2 previous biopsies had not been sent for histology because the family could not afford it. It was also not feasible to stage this patient with a CT scan as it was such a costly investigation and it would not have changed the clinical stage or altered management of her disease. Given the contiguous nature of HL spread, it is likely she had extensive lymphadenopathy. We were able to demonstrate a good response to chemotherapy by repeating the trephine. In the absence of second line therapy, staging and follow-up scans are less relevant, although one might have considered local radiotherapy if there had been bulky disease at presentation on CT or a localised residual mass following chemotherapy.

The third issue is that of treatment availability, feasibility and cost. Combination chemotherapy with doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) is the standard treatment for advanced stage HL in developed countries but bleomycin and dacarbazine are not available in Ghana. CHOP chemotherapy was used instead as all the drugs were available. These drugs are included in many HL regimens including ChlVPP/PABLOE, ChlVPP/EVA and Stanford V (Johnson *et al*, 2005; Hehn & Miller, 2004). Within the department the nurses also had some experience of giving CHOP and looking after patients with CHOP chemotherapy induced side effects.

2 further cases of HL were treated with CHOP – the first presented with lymphadenopathy without marrow involvement and initially responded well to CHOP, but then her lymphadenopathy started to progress between cycles of chemotherapy. The second patient presented as an inpatient, performance status 3, with a five month history of breathlessness, oedema and fever. He had cervical lymphadenopathy and pancytopenia. HL was confirmed on bone marrow trephine and lymph node biopsy and he was given CHOP chemotherapy as an inpatient. He initially improved and was discharged, but then died at home, probably from neutropenic sepsis, prior to his second cycle of chemotherapy.

In summary, patients in the project with HL tended to present late and have advanced disease at presentation. Full staging was not possible as it was too expensive for patients to afford and conventional HL treatment was not available. Despite this the patient described responded well to treatment although remission duration is not yet known.

Case 2

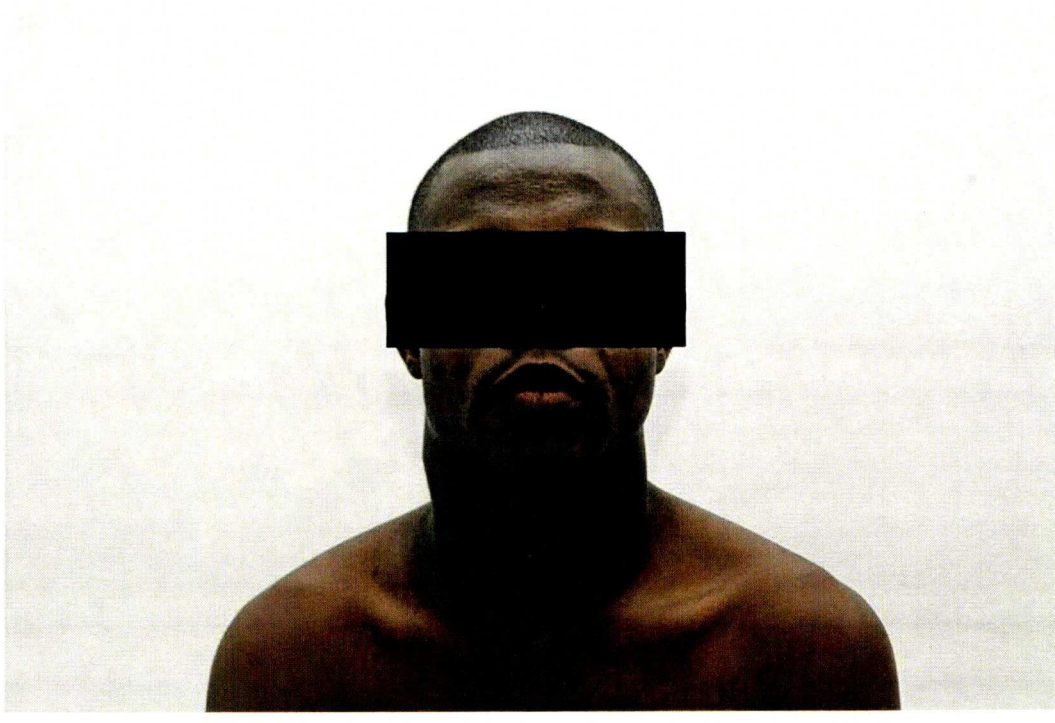
Case summary

30 year old presented with a 14 month history of a mass in the right side of his neck, but no systemic symptoms. Clinically a malignancy was suspected, but the lymph node biopsy demonstrated the typical morphological features of sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease).

Presentation

A 30 year old, single, male, trader presented to the surgical outpatients at KATH in October 2004 with a 3 month history of a right neck mass. In July 2004 he had developed a right sided headache and attended a local hospital. He was given an intramuscular gluteal injection of analgesia. The following day noticed a mass on the right side of his neck. He was otherwise well with no fevers, sweats or weight loss. The nodal mass in his neck was biopsied in October 2004 and reported by a private laboratory as showing sinus histiocytosis. He was therefore referred to the haematology outpatient department and entered into the LPD study on 13th September 2005. He had bilateral cervical nodes the largest measuring 7x3cm on the right. He also had an enlarged right supraclavicular node measuring 2x4cm. (Figure 5.4)

Figure 5.4 – Case 2 Rosai Dorfman - Bilateral cervical lymphadenopathy



Investigation results

His blood results were unremarkable: haemoglobin 13g/dl, WCC 5.3x109/l, platelets 346x109/l, ESR 48mm/min and HIV test negative. No radiological investigations were performed. Bone marrow aspirate and trephine and a repeat biopsy of the neck mass were sent to HMDS. The bone marrow showed reactive changes, but no evidence of infiltration and the neck biopsy confirmed sinus histicytosis. The capsule in places showed marked thickening. Overall lymph node architecture was generally preserved. There were numerous small B-cell follicles. The most striking feature however, was that of sinus histiocytosis, expressing CD68 and s100 protein. (Figures 5.5-5.8) A number of the histiocytes were actively phagocytosing lymphocytes, plasma cells and granulocytes. The features were considered to be consistent with sinus histiocytosis with massive lymphadenopathy (Rosai Dorfman).

Figure 5.5 – Case 2 Rosai Dorfman – H&E x10 magnification showing prominent sinus histiocytosis

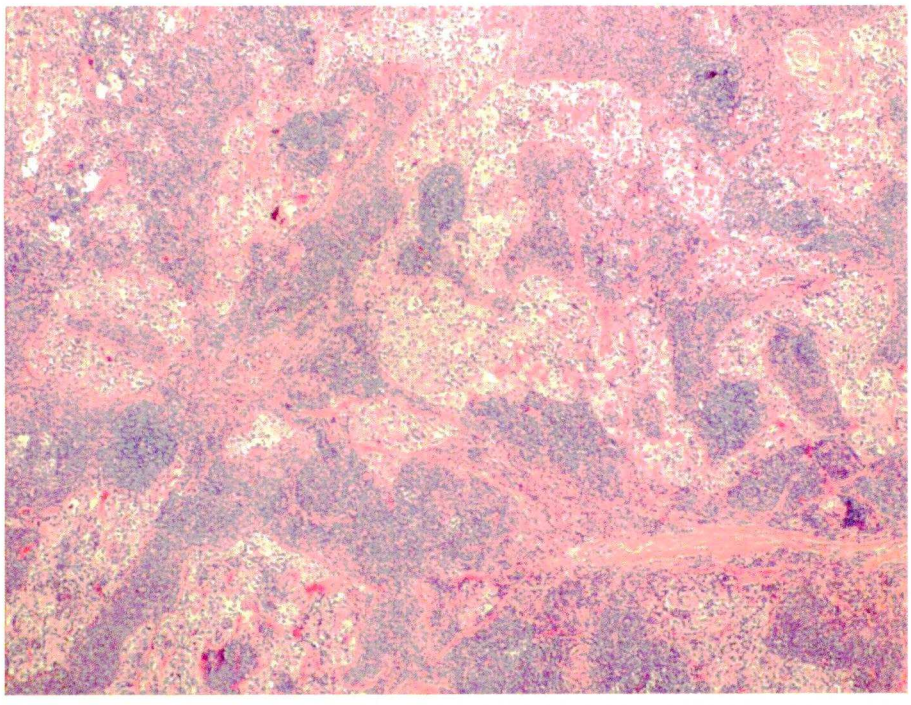


Figure 5.6 – Case 2 Rosai Dorfman – H&E x60 magnification showing histiocytes phagocytosing lymphocytes and plasma cells

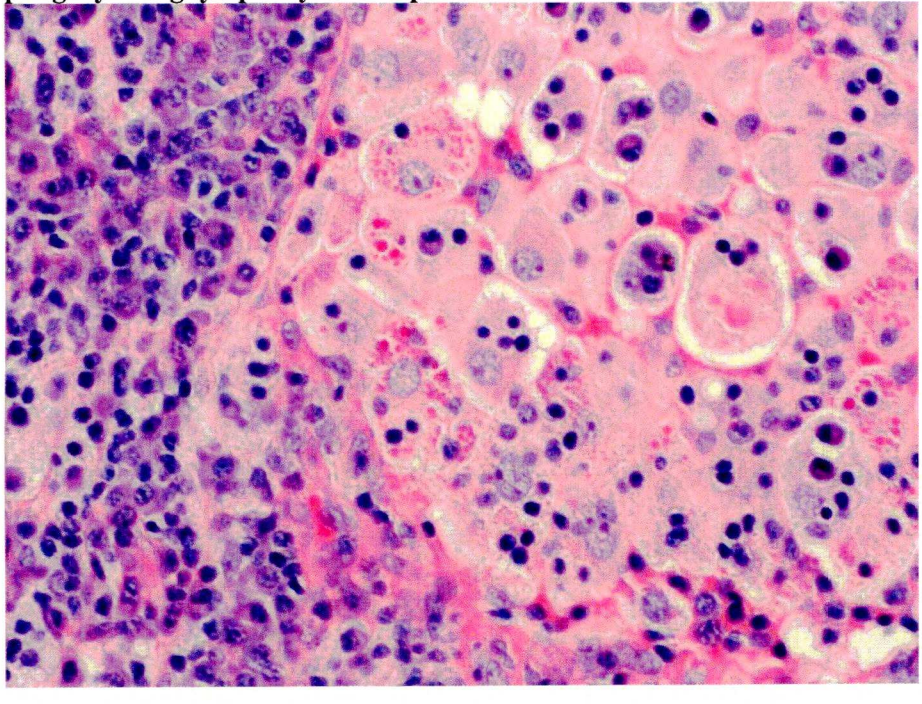


Figure 5.7 – Case 2 Rosai Dorfman – Immunohistochemistry s100 protein

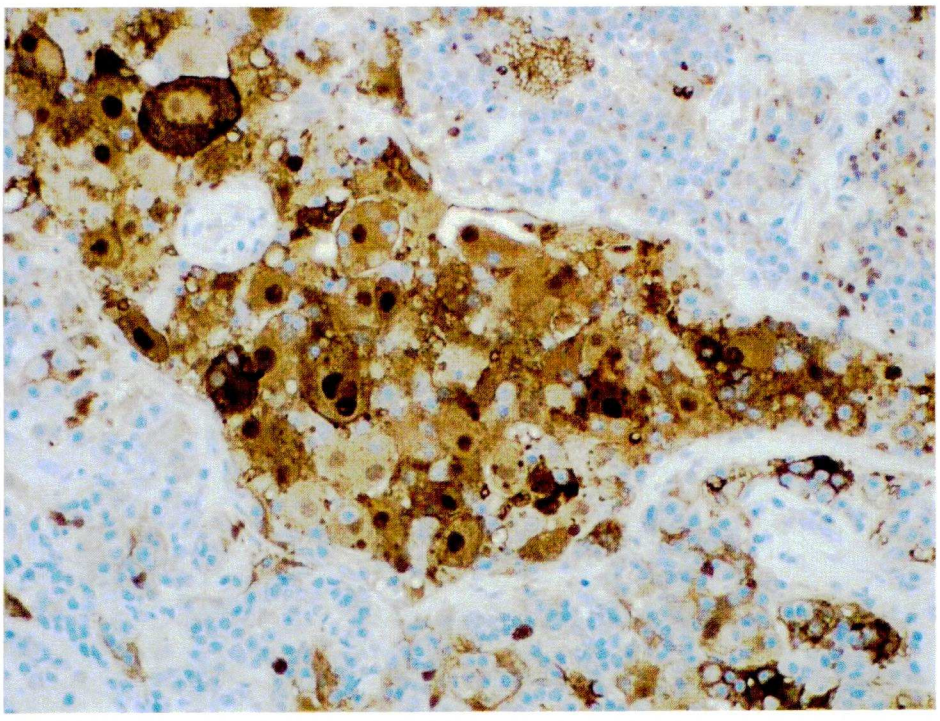
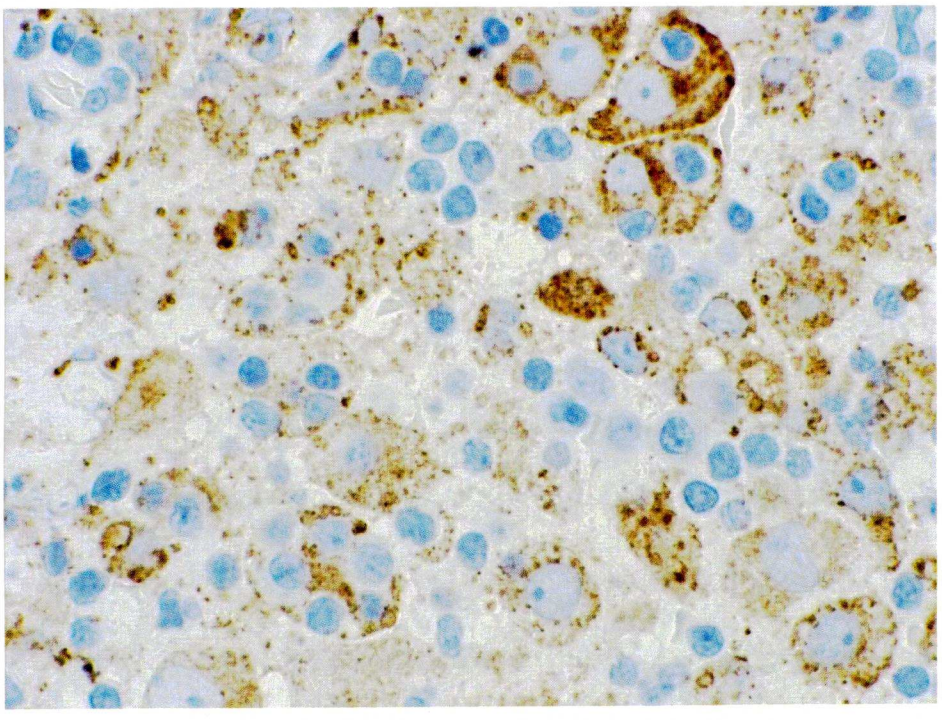


Figure 5.8 – Case 2 Rosai Dorfman – Immunohistochemistry CD68



Treatment

The diagnosis was explained to the patient and the lymph node mass started to slowly decrease in size. No specific treatment was necessary and the patient was lost to follow-up in January 2006.

Discussion

Sinus histiocytosis with massive lymphadenopathy (SHML), or Rosai Dorfman syndrome, is a benign, usually self-limiting disorder of unknown aetiology that was first described in 1969 (Rosai & Dorfman, 1969). It typically affects children and young adults, but can occur at any age. It commonly presents with massive, bilateral, painless cervical lymphadenopathy with or without involvement of other nodal sites. Extranodal involvement also occurs in almost half the cases. There is often associated fever and occasionally weight loss. SHML does not usually require any treatment, but can be recurrent or progressive and in rare cases can be fatal. Treatment options, where necessary, include steroids, chemotherapy, radiotherapy, excision, antibiotics and other agents such as thalidomide and α-IFN, however responses are variable (Pulsoni *et* al, 2002; Tjiu *et* al, 2003; Hargett & Bassett, 2005; Aouba *et al*, 2006; Ocheni *et al*, 2007; Pagel *et al*, 2007; Utikal *et al*, 2007).

Patients with SHML often have a reactive blood picture with a raised ESR, anaemia, neutrophilia and polyclonal hypergammaglobulinaemia. The marrow is not usually involved. Histology is crucial for diagnosis - there is typically marked fibrosis in the capsular and peri-capsular areas and distension and engorgement of medullary and subcapsular sinusoids by phagocytic histiocytes. Other features of SHML include lymphophagocytosis and erythrophagocytosis by histiocytes in the lymph node sinus. The active histiocytes are positive for S100 protein, CD11c, CD14, CD33, CD68, acid phosphatase and non-specific esterase (Eisen *et* al 1990, Paulli *et al* 1992).

SHML can therefore clinically mimic a malignant process and highlights the importance of making a definitive diagnosis before starting treatment with chemotherapy and radiotherapy with associated cost and side effects. The local private laboratory had

already made the correct diagnosis and this was confirmed on repeat biopsy. Since his nodes were so large it was difficult to convey the benign nature of the disease to him and as they were also so visible they were making it difficult for him to find work. A further concern was that he would not understand that no treatment was necessary and would get frustrated with our watch and wait plan and go to a local herbal practitioner instead with associated costs and side effects. This may have happened as the patient was lost to follow-up.

Case 3

Case summary

18 year old presented with a 14 month history of fluctuating bilateral neck nodes. FNA was not diagnostic. Biopsy showed EBV-associated NPC. The patient responded to radiotherapy and went on to have consolidation chemotherapy under the care of the oncologists.

Presentation

An 18 year old, single, male, student was referred from his local district hospital to KATH polyclinic in May 2005. In April 2004 he had felt generally unwell and noticed a pain in the right side of his neck. This resolved with antibiotics; however in June 2004 he noticed a swelling in the left side of his neck. This persisted despite antibiotics. In January 2005 a punch biopsy of the left neck node was attempted, but only skin was biopsied. He was referred from polyclinic to the haematology outpatients and was seen in June 2005. He had two large left cervical nodes measuring 4x4cm and 3x3cm. He had no fevers or sweats and only mild weight loss.

Investigation results

He was slightly anaemic with haemoglobin 10.7 g/dl and LDH was raised at 517 U/l as was ESR at 88 mm/min. Other blood results were normal and HIV test was negative. Chest xray was normal and an FNA of the left neck node showed no malignant cells. Bone marrow aspirate and trephine showed no evidence of infiltration. Lymph node

biopsy was performed on 21st June 2005 and showed EBV associated NPC. There was extensive replacement by metastatic tumour consisting of cohesive sheets of tumour cells with large oval nuclei and prominent eosinophilic nucleoli. (Figure 5.9) There was no evidence of CD45 expression and epithelial origin was confirmed by demonstrating expression of the pan-cytokeratin MNF116 although both CK7 and CK20 were negative. (Figure 5.10) Expression of EBV-associated latent membrane protein-1 (LMP-1) was demonstrable in a significant proportion of tumour cells. (Figure 5.11)

Figure 5.9 – Case 3 Nasopharyngeal carcinoma – H&E x40

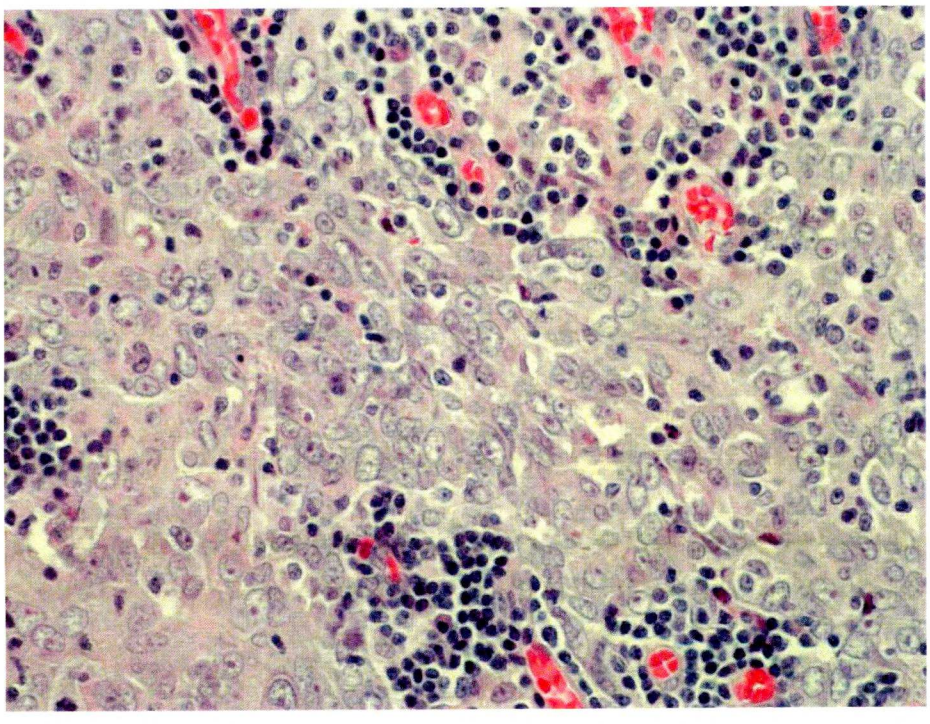


Figure 5.10 – Case 3 Nasopharyngeal carcinoma – Immunohistochemistry MNF116

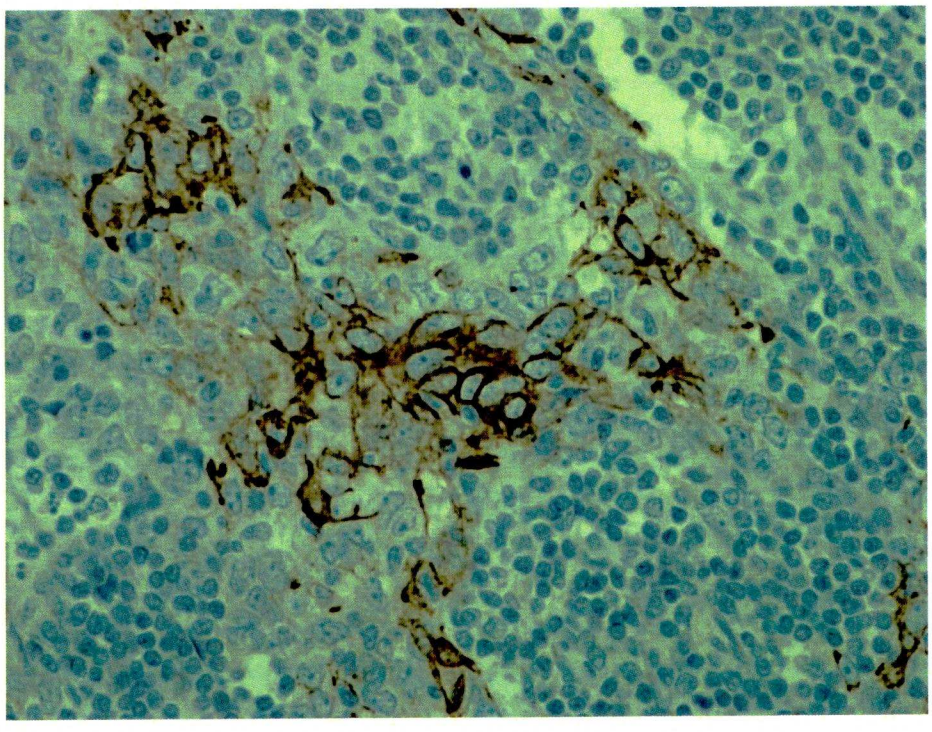
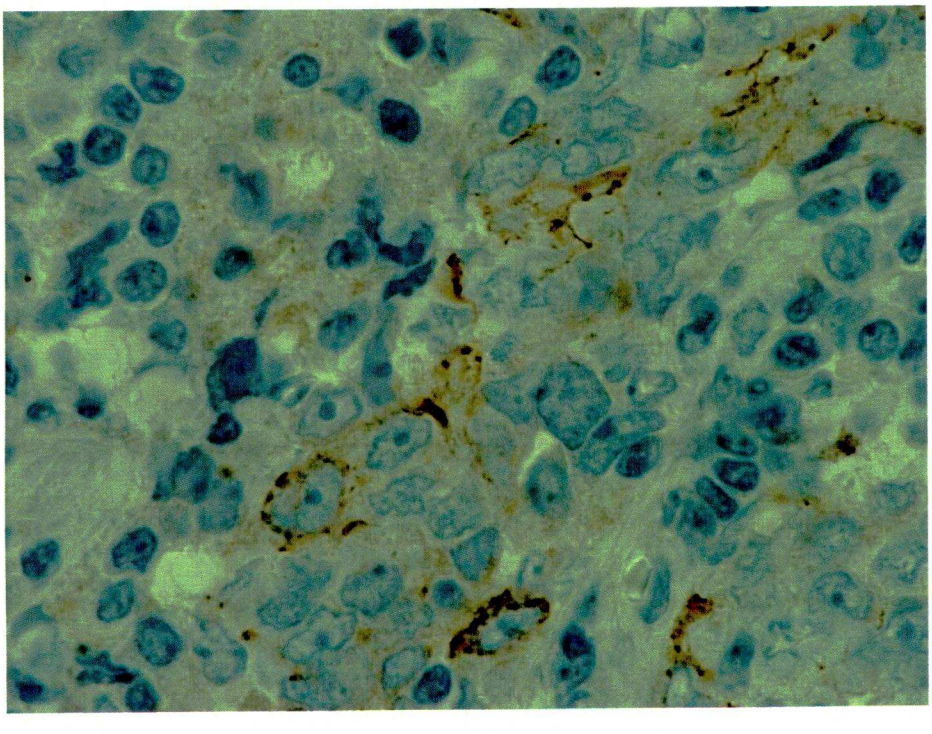


Figure 5.11 – Case 3 Nasopharyngeal carcinoma – Immunohistochemistry LMP-1



Treatment

The diagnosis was explained to the patient and he was referred to the oncologists for local radiotherapy. He responded to this and went on to have chemotherapy with 5-fluorouracil and cisplatin.

Discussion

NPC is a highly radiosensitive and chemosensitive tumour that develops from the epithelium of the nasopharynx, usually originating in the fossa of Rosenmuller (Tao & Chan, 2007). It has a striking geographical and ethnic distribution worldwide being particularly prevalent in Southern China and southest Asia, especially in the Cantonese 'boat people' where the incidence of NPC is 54.7/100 000/year (Tao & Chan, 2007). The incidence in non-high risk populations is less than 1/100 000/year. Some North African populations (Algeria, Morocco and Tunisia) fall into higher risk groups, but other African populations are not known to be high risk (Yu & Yuan, 2002). The M:F ratio in most populations is approximately 2-3:1 and incidence usually increases with age until a peak between 45 and 54 years (Yu & Yuan, 2002). Increased incidence of NPC occurs in populations where there is consumption of preserved foods in early childhood and a move away from this practice has been linked with a fall in incidence (Yu & Yuan, 2002).

A clear link with EBV in tumour pathogenesis has also been demonstrated and was first described in 1966 (zur Hausen *et al*, 1970). Further work suggests a multistep process in the pathogenesis and development of NPC, involving a combination of genetic susceptibility, EBV infection and upper respiratory tract infection and inflammation (Tao & Chan, 2007). Subsequent defects of apoptosis and DNA repair results in the transformation of a pre-malignant lesion (carcinoma in situ) to a malignant NPC (Tao & Chan, 2007).

NPC was low on the list of differential diagnoses in this patient prior to the biopsy. This demonstrates the importance of being able to make accurate and timely histological diagnoses in such patients so that appropriate treatment can be given. If the project had not been available when he presented potential options would have included:

1) Observation with a view to repeat FNA resulting in a delay in diagnosis;
2) Referral to the surgeons for a biopsy with the sample sent to a private laboratory in Accra again resulting in a delayed and costly diagnosis;
3) Empirical therapy for presumed tuberculosis or lymphoma – neither of which would have been the correct management for his diagnosis.

Case 4

Case summary

30 year old presented with a 1 month history of right neck swelling. FNA of the node was suggestive of lymphoma, but lymph node biopsy showed granulomata. The patient was referred to the tuberculosis clinic for treatment.

Presentation

A 30 year old, single, female, trader presented to the ear, nose and throat department at KATH on 19th January 2006 with a 1 month history of enlarged right neck nodes. She also reported having lost weight 1 month previously, but had already regained this and had no fevers or sweats. On examination she had 2 posterior cervical nodes on the right side of her neck, but no other palpable nodes. She had no past medical history of note, but had been taking herbal medications until 1 week previously.

Investigation results

Blood results were fairly unremarkable with haemoglobin 11.4g/dl, WCC $8.3x10^9$/l, platelets $270x10^9$/l, LDH 152 U/l, although ESR was raised at 120 mm/min. FNA of one of the enlarged nodes was performed on 23rd January 2006 and showed lymphocyte proliferation and several large cells with bluish nucleoli and reddish cytoplasm. The differential diagnosis was reported as being HL or NHL. The patient was entered into the study on 31st January 2006 and a lymph node biopsy was performed on 11th February 2006. This showed coalescent necrotising granulomata. (Figures 5.12 and 5.13) There was no evidence of malignancy. Although Ziehl-Neelson stains were negative the

findings were consistent with mycobacterial infection. The bone marrow showed extensive necrosis. No radiological investigations were performed.

Figure 5.12 – Case 4 Tuberculosis – H&E x10

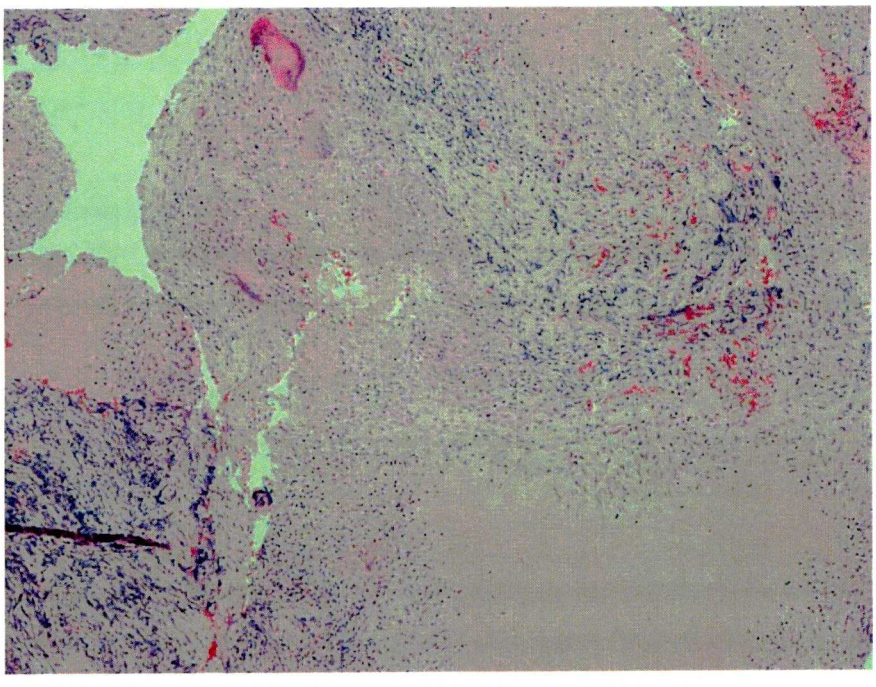
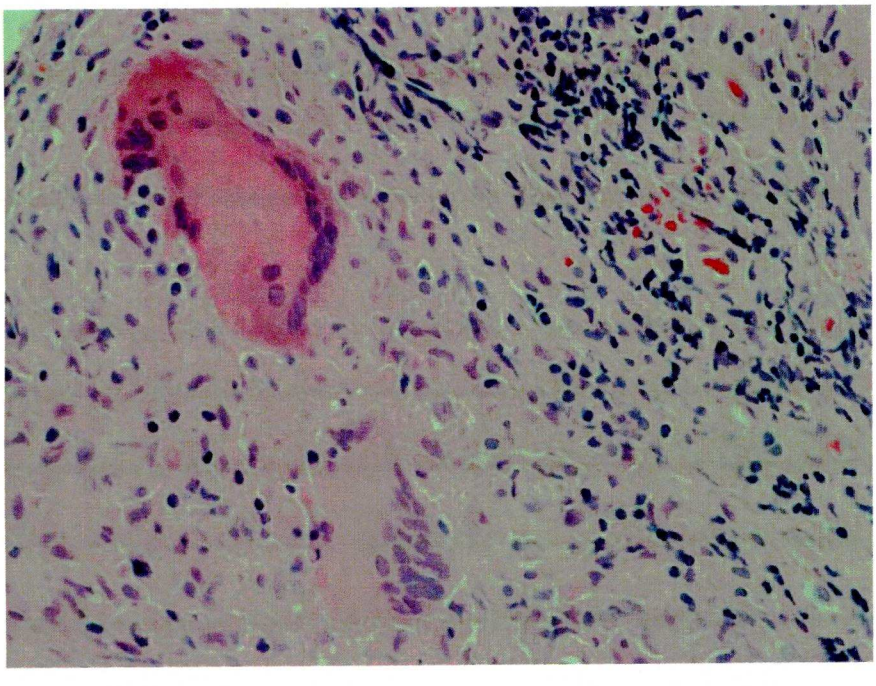


Figure 5.13 – Case 4 Tuberculosis – H&E x40



Treatment

The patient was referred to the tuberculosis clinic for treatment and did not receive any chemotherapy.

Discussion

This case shows how important it is to make the correct diagnosis prior to commencing treatment and that histological diagnosis is crucial in the diagnosis of suspected LPDs. In this study of 150 patients suspected to have a LPD we diagnosed 4 cases of tuberculosis. 2 of these cases had had an FNA – the first, described above, was suggestive of a LPD, the second was not diagnostic. The 2 cases that did not have an FNA were clinically felt to have a LPD. They both had large disseminated nodal masses, one patient was HIV positive and the other, at 83 years, was the oldest patient in the study. Clinicians in Ghana have a great deal of experience diagnosing tuberculosis clinically and so patients that were felt by local clinicians to have tuberculosis, as opposed to a LPD, were not entered into the study.

Our study also included a patient that had had a lymph node biopsy reported locally as showing granulomatous lymphadenitis. The patient had been treated with anti-tuberculosis treatment with no improvement in her symptoms or lymphadenopathy. A repeat biopsy reported at HMDS showed HL. We were unable to obtain the original block to verify whether there had been dual pathology accounting for her symptoms and signs. This seems unlikely, however, as the patient had deteriorated on anti-tuberculosis medications, but responded to CHOP chemotherapy.

It is known that dual pathologies concerning LPDs and tuberculosis can and do occur, especially in areas with a high prevalence of tuberculosis (Fangourgiakis *et al*, 2008). This means that patients diagnosed as having a LPD in such settings must be observed carefully and if dual pathology is confirmed or suspected they need to receive anti-tuberculosis treatment with their chemotherapy. Anti-tuberculosis treatment in Ghana is free and well supervised to improve compliance.

The reverse is also true and the patients diagnosed as having tuberculosis need to be followed up carefully to ensure that their nodes and symptoms improve with the anti-tuberculosis treatment. If they do not improve further biopsy is indicated. This is difficult in a setting where so many patients are lost to follow-up and it is not feasible for patients to remain under follow-up in two clinics due to the cost and travelling that would involve for the patient. Education of both clinicians treating tuberculosis and lymphomas and patients is therefore important.

This patient's biopsy was negative on Ziehl-Neelson stain, but had other features consistent with tuberculosis. This is a common problem as Ziehl-Neelson stain is only positive if there are approximately 10^4 to 10^6 bacteria per milliliter of tissue specimen; PCR on tissue blocks can be used to increase the sensitivity of diagnosis (Li *et al*, 2000).

Case 5

Case summary

67 year old with hepatosplemomegaly and peripheral blood lymphocytosis. This case illustrates how the model we developed facilitated making a complex diagnosis.

Presentation

A 67 year old, married, female, farmer presented to KATH polyclinic on 24th March 2005 with abdominal pain, loose stool, sweats and weight loss. Spleen was palpable 19 cm below the left costophrenic margin and the liver was palpable 6cm below the right. She was initially treated with proguanil, folic acid and trenxene (an analgesic). She was referred to the haematology outpatient department and entered the LPD study on 20th April 2005.

Investigation results

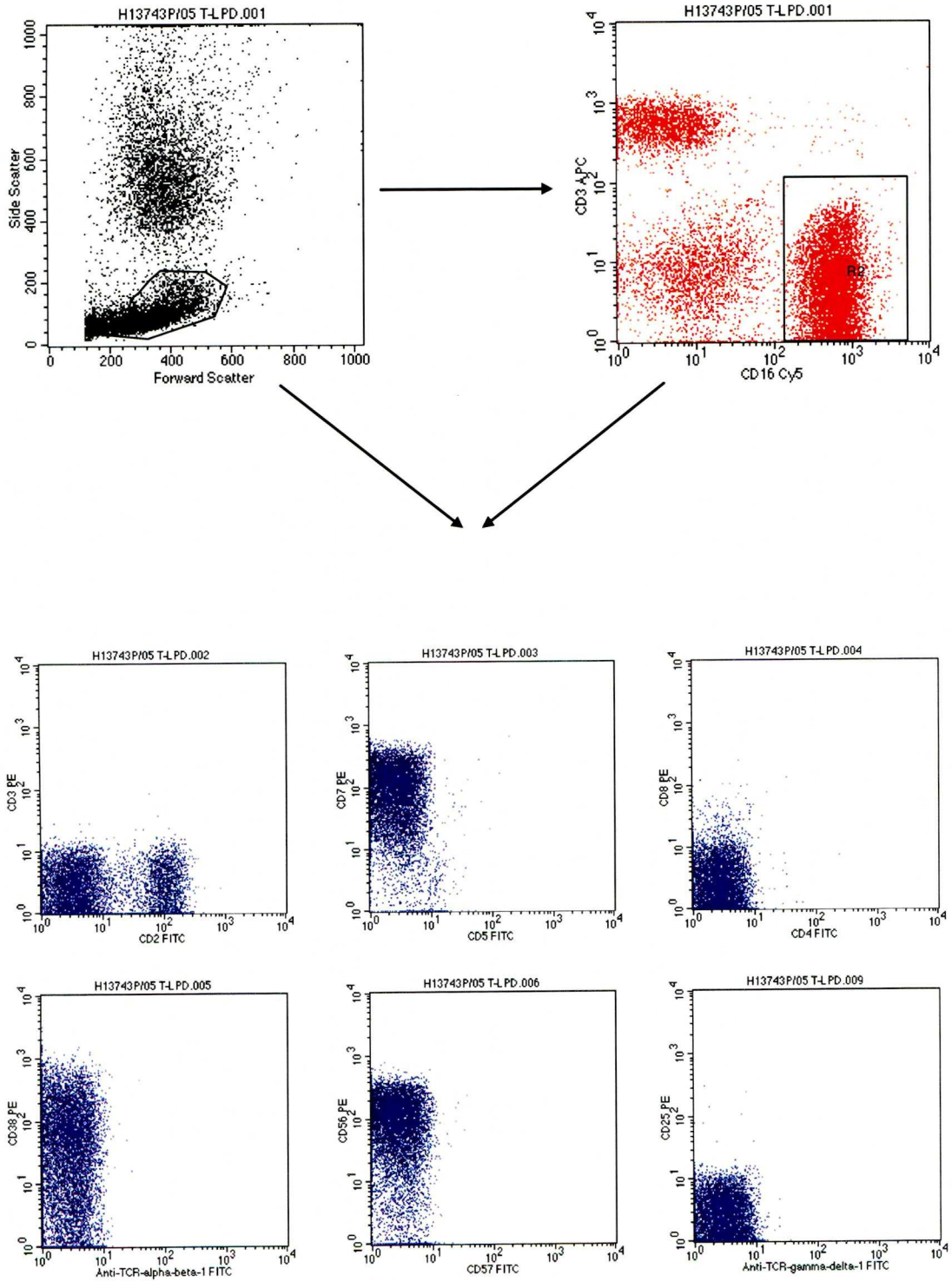
Blood results performed locally showed: haemoglobin 9.2g/dl, WCC $30.4x10^9$/l, platelets $151x10^9$/l, MCV 89.8fl, neutrophils $6.04x10^9$/l, lymphocytes $22.9x10^9$/l, LDH 266U/l and HIV test was negative. The blood film showed a marked lymphocytosis with mainly

small to intermediate sized cells with a high nuclear:cytoplasmic ratio and little evidence of cytoplasmic granulation. There was also marked polychromasia with some nucleated red blood cells. Bone marrow trephine reported at HMDS showed increased cellularity for age, normal haemopoietic elements were present as well as a striking T-cell lymphocytosis. There was an atypical lymphoid infiltrate with a highly aberrant immunophenotype. No definitive diagnosis was made on the trephine, but additional flow cytometric analysis performed on peripheral blood taken to HMDS in July 2005 demonstrated the following immunophenotype: CD3− CD16+ CD2+/− CD7+ CD5− CD8− CD4− CD38+ TCR− CD56+ CD57− HLADR− CD52+ CD1a− CD34−. A diagnosis of NK-cell leukaemia was made. (Figure 5.14)

Treatment

Whilst the diagnosis was being clarified the patient continued treatment with proguanil as the differential diagnosis was HMS. She did not respond to this however and at 6 month follow-up the spleen remained 19 cm. Follow-up bloods showed: haemoglobin 8.2g/dl, WCC $21.6x10^9$/l, platelets $85x10^9$/l. She became more symptomatic of her splenomegaly and so was given a trial of oral cyclophosphamide. After 2 cycles she felt better, but the spleen was still palpable at 19 cm and the haemoglobin and platelets had fallen further: haemoglobin 7.5 g/dl, WCC $10.1x10^9$/l and platelets $37x10^9$/l. As her counts had fallen, her spleen size had not reduced and the chemotherapy was expensive for a family on such limited income a period of watchful waiting was commenced. No further follow-up is available.

Figure 5.14 –Case 5 NK cell leukaemia – Flow cytometry – gated on CD3 negative CD16 positive cells



Discussion

This case demonstrates that the model we developed assisted not only in straightforward cases, but also in making less common, more complicated diagnoses. Chronic NK-cell leukaemia makes up approximately 5% of all large granular lymphocyte disorders and so is a rare LPD (Lamy & Loughran, 2003). Treatment decisions in this case were difficult, as will be discussed, and knowing the diagnosis helped in making these decisions. As with many indolent LPDs, the decision to commence treatment was largely based on the patient's symptoms, although her blood counts had also started to fall. Such subjective decisions are particularly difficult to make when communication is via an interpreter and where patient involvement in treatment decisions is less part of the culture than it is in the UK.

Treatment options for indolent NK cell leukaemia include cyclophosphamide, methotrexate and ciclosporin (Alekshun & Sokol, 2007). We decided to treat her with oral cyclophosphamide as this was readily available and frequently used in the haematology outpatient clinic. Oral methotrexate was occasionally, but not reliably available. Ciclosporin was not available locally and there were no facilities for checking therapeutic levels.

The patient and her husband were farmers living in a rural village and did not have much money, but were able to pay for the chemotherapy. It is likely in such a situation that the extended family helped to cover the cost of this. Following the 2 cycles of chemotherapy her counts had deteriorated further, although she was feeling better. In a situation where it is not possible to give supportive care with platelet transfusions it was very important not to drop her counts any further with chemotherapy.

There may have been a significant placebo effect regarding the cyclophosphamide as her spleen did not improve and her counts deteriorated. The patient looked and reported feeling better following the 2 cycles of cyclophosphamide however. Again it was very difficult to assess this via an interpreter.

A treatment option that we considered was splenic radiotherapy. This may be a useful treatment modality in the future for symptomatic, indolent, splenic LPDs in a topical setting as radiotherapy becomes more widely available. Radiotherapy is often quite expensive however, but less risky in such a setting than splenectomy. A typical course of splenic radiotherapy would cost approximately 1 million cedis (£60).

Summary

- Chemotherapy and radiotherapy are available in developing countries, such as Ghana, so it is vital that facilities for the rapid and correct diagnosis of LPDs are also available so that the correct treatment is given
- The possibility of dual pathology or one pathology mimicking another, especially tuberculosis and lymphoma, needs to be borne in mind and physicians educated accordingly
- Using this method of remote diagnosis, it was possible to make complicated and clinically unexpected diagnoses thus improving patient management

Chapter 6 - Discussion

Introduction

This chapter summarises and describes the limitations of the project, highlights potential pitfalls in the diagnosis of lymphomas, looks at important issues relating to research in developing countries and finally discusses future research potentials.

Project summary

The project achieved its main objective which was to develop and test a model for the diagnosis and classification of LPDs in Ghana, West Africa. Utilising modern laboratory and communication methods, we were able to set up a system to diagnose and categorise LPDs in Ghana. Modern diagnostic techniques, not currently available locally, were used in a clinically meaningful way and results were used to inform treatment decisions. Preliminary epidemiological data regarding LPDs in West Africa was also collected with a view to expanding on this in future studies. The system was robust and is sustainable.

There were significant differences between the spectrum of LPDs diagnosed in Ghana and those diagnosed at HMDS. In the project there were significantly more cases of SMZL, PTCL and T-ALL, but less cases of DLBL than seen at HMDS. Amongst the patients with SMZL there was a striking excess of female patients. We envisaged that the excess numbers of cases of SMZL seen in Ghana compared to Yorkshire would be due to chronic infection causing antigenic stimulation. We did not see an excess of cases with mutated *IGHV* genes, and in those cases that were mutated, the mutation load was relatively low. Further work in this area is necessary.

Limitations of the project

The problems encountered when setting up the project are discussed in Chapter 2. These included the differences between the two centres, the number of patients lost to follow-

up, communication problems, missing results as patients were unable to pay for all the relevant investigations, potential concerns regarding the quality of local test results and poor documentation. Where possible ways around these problems were sought in order to minimise their impact on the project outcomes, however this was not always possible for example it was not possible to do all the usual staging investigations on patients and this resulted in missing data. Further data is missing as a result of patients being lost to follow-up both before and after a definitive diagnosis was made.

150 patients were entered into the project over a 13 month period. Although all the patients entered were felt to clinically have a LPD only 41% were diagnosed with a LPD. This resulted in small numbers of patients in each LPD sub-group making comparisons difficult.

As a result it is not possible to draw major epidemiological conclusions however this project does provide the basis for further work investigating LPDs in West Africa.

Potential pitfalls in the diagnosis of lymphoma

Problems regarding accurate lymphoma diagnosis are not limited to developing countries. The clinical impact of expert pathological review on lymphoma management in Wales was assessed between January 1998 and August 2000 (Lester *et al,* 2003). Following expert review by the All Wales Lymphoma Panel (AWLP), the pathological diagnosis, made by local district general hospital pathologists, was altered in 125 of 745 (17%) specimens. Case notes were reviewed in 99 (79%) of these cases. In 46 of the 99 cases the patients' management was changed as a result of expert pathological review. Overall, management was changed in 8% of cases referred to AWLP for expert central pathological review.

As discussed in Chapter 1, five ILSG pathologists in Germany remotely reviewed 206 cases of NHL diagnosed in Kuwait (Temmim *et al*, 2004). In 4 cases (2%) the diagnosis made by the local pathologists was altered when the case was reviewed and in a further 4

cases the review material was not adequate. Although this study was done as part of the 'Clinical characteristics and pathological classification of NHL in the developing countries' project the original diagnoses were made in a national centre with facilities for immunophenotyping. It is therefore not a true reflection of the facilities available when most cases of NHL are diagnosed in developing countries hence in the low discordance rate.

Immunophenotyping is crucial in the modern diagnosis of LPDs. Our study demonstrates that it is feasible to perform this remotely in a clinically meaningful manner. Performing such tests remotely in an established laboratory such as HMDS has some advantages such as the economy of scale, a high throughput allows batching of tests which reduces the reagent costs, and makes it easier to maintain a high quality service. Ideally a transfer of technology should occur so that these tests become available locally to provide a more flexible service, avoid the costs and potential problems associated with transportation and allow local scientists and clinicians to learn new techniques. These techniques are expensive and this is currently prohibitive for most patients in countries like Ghana. It is however crucial to ensure that the correct diagnosis is made before potentially harmful therapies such as radiotherapy and chemotherapy are given.

Research in developing countries

In 1990 the Commission on Health Research for Development estimated that only about 5% of the world's resources for health research were being applied to the health problems of developing countries, where 93% of the world's burden of 'preventable mortality' occurred (Health Research, Essential Link to Equity in Development, 1990). In 1998 the Global Forum for Health Research was founded to try to address this imbalance which was described using the term '10/90 gap'. Non-communicable diseases now have a much greater impact in developing countries making it difficult to estimate what percentage of the world's resources for health research is currently spent in developing countries. There remains a gross imbalance, however, and although the term '10/90 gap' is no longer accurate it continues to be used to describe this.

Although more health research is desperately needed in developing countries, carrying out such research needs to be carefully planned to avoid exploitation of participants and other potential ethical issues. Emanuel *et al* (2004) outline a framework for ethical research in developing countries that involves 8 principles with 31 associated benchmarks. (Appendix 9) These principles and benchmarks encompass the key elements in the development of a collaborative diagnostic and therapeutic service described by Eden (2002) and discussed in Chapter 1.

Collaborative partnership, the first of the 8 principles, is crucial to ethical health research in developing countries. As discussed in Chapter 1, collaborations between developing and more developed partnerships are an excellent way of transferring diagnostic skills and techniques as well as facilitating research and improving the diagnosis and treatment of certain illnesses. When collaborative research is set up, it is important that local collaborators lead the decision making process so that the most appropriate research takes place and there is a transfer of appropriate skills and knowledge.

A long term, sustainable approach to research projects is necessary and short term 'fixes' must be avoided (Lawn & Lawn, 2002). This is vital in order to avoid diverting trained healthcare staff from clinical work into short-term projects and adding to the 'brain drain' that already exists in healthcare in many developing countries. Transfer of relevant health technology in an appropriate manner is necessary to build local scientific capacity to monitor and control disease; for example the Sustainable Sciences Institute in San Francisco aims to help infectious disease biomedical scientists in developing countries access training, funding, information, collaborators, equipment and supplies (Harris & Tanner, 2000). The institute emphasises long term follow-up of technical, financial and material support via 4 programmes: on-site training, small grant, networking and consulting and material aid.

Ensuring informed consent of participants can be difficult in any research project, but language and cultural barriers can make this even harder to achieve. Hill *et al* (2008)

looked at the outcome of informed consent for a placebo-controlled vitamin A supplementation study among Ghanaian women aged 15-45 years. They surveyed 1971 trial subjects and found that although subjects knew they were taking part in research, most thought they were receiving active and beneficial medication. Variables associated with knowledge were education and district of residence, but fieldworkers' characteristics were not associated with subjects' knowledge.

Quality assurance is another issue common to research projects throughout the world, but is a particular concern in developing countries, where such systems may be non-existent or poorly implemented. Scott *et al* (2007) set up a simple and sustainable quality assurance program for clinical chemistry services in Eritrea. Over a 10 year period they increased the number of tests available locally and provided training and assistance with quality control. They encountered many difficulties during this process, such as the withdrawal of spare parts and reagents by a US based manufacturer and a lack of e-mail or fax communication with the district hospitals in Eritrea. When quality control failures occurred they were investigated – at one site recurrent failures over a 6 month period were found to be due to poor-quality water for cleaning glassware and reuse of disposable cuvettes and reagent boats. If such measures are not in place in laboratories then this has serious implications for the interpretation of results generated by that laboratory.

Future research

As discussed in Chapter 1, prospectively collected data utilising the WHO classification of LPDs was not previously available in sub-Saharan Africa. We were only able to get a 'snapshot' of information regarding LPDs in Ghana as most of population does not have access to health care, however it will being interesting to observe whether there are any changes in the patient demographics or the diagnoses made in the next few years as more patients are able to access health care in Ghana as a result of the national insurance system. For example, with more equitable access to health care younger patients, who previously struggled to pay for hospital visits, investigations and treatment as they often did not have access to the 'extended family' system, may present earlier and more

frequently resulting in, for example, earlier presentation of patient with HL and perhaps more patients presenting at a stage where diagnosis and treatment is more successful rather than presenting with end stage disease, so frail that treatment is futile. Ongoing data collection will also provide valuable epidemiological information regarding LPDs in Ghana which may influence future treatment of LPDs both in Ghana and potentially elsewhere.

Ongoing improvements in technology such as e-mail and telepathology, along with faster and more reliable transportation of samples, as well as improvements in techniques for stabilizing specimens, will combine to make real-time diagnostic collaborations more feasible and clinically relevant.

Chapter 7 – Conclusion

There is evidence that the spectrum of LPDs in Ghana, and other West African countries, is likely to be different to that found elsewhere. Infection may play a role in pathogenesis and this is likely to influence treatment options. With the increasing availability of chemotherapy and radiotherapy in Ghana, and other developing countries, there is an urgent need to improve the diagnosis of LPDs, and other malignancies, to ensure that appropriate treatment is given. Real-time collaborative diagnosis alongside research, with a view to transfer of diagnostic skills and technology, would address these problems in a practical and sustainable manner.

A collaborative model was set up to comprehensively diagnose LPDs in Ghana. Local clinicians and technicians were closely involved in the design and running of the project with a view to the model being continued when the initial MD project finished. Full diagnostic work up was performed on samples sent to the UK and the results were available to clinicians in Ghana in a clinically relevant time span. Transfer of skills and technology is envisaged over the coming years to improve the diagnostic facilities available locally.

It was possible to remotely diagnose and classify patients presenting with a suspected LPD in a clinically relevant time scale. The use of state of the art diagnostic techniques improved diagnosis and the management of 46/150 (31%) patients, even in a setting where there were limited therapeutic options. 41% of patients (61/150) with a suspected LPD had a proven LPD, 13% (20/150) had other diagnoses and in 46% (69/150) there remained some diagnostic uncertainty.

This study has demonstrated that it is possible to perform complex immunophenotypic, cytogenetic and molecular analysis as part of the diagnostic work up of patients with suspect LPDs in West Africa. This small cohort of patients has provided an insight into the spectrum of LPDs seen in Ghana. Further study is clearly worthwhile and, utilising

the model we have developed, it should be possible to make a definitive assessment of the epidemiology of LPDs in Africa and gain insight into their biology.

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Appendix 1: Laboratory techniques in the diagnosis of LPDs

Histology

Biopsy samples need to be fixed and processed prior to staining. If it is anticipated there will be minimal delay between a biopsy sample being taken and it arriving at the laboratory, it is sent fresh so that flow cytometry can be performed, allowing rapid immunophenotyping results. The biopsy is then fixed in 10% formalin. If it is not possible to get the biopsy sample to the laboratory quickly it is sent in 10% formalin. Formalin is an effective fixative, but needs to be allowed to penetrate the biopsy specimen therefore for larger biopsies the sample is sliced before fixing in formalin. Samples are fixed for 24-48 hours before being processed in a stepwise manner which allows replacement of water with graded alcohols which are then replaced by chloroform or xylene. This allows permeation by paraffin wax, forming a tissue block. As bone is harder than paraffin wax, bone marrow trephine samples need to either be decalcified before being paraffin embedded or embedded in resin, which is harder than paraffin. As trephine morphology is better preserved with resin embedding this is the method used at HMDS.

Immunophenotyping

Flow cytometry

Samples

Peripheral blood and BM aspirate samples can be kept in EDTA for up to 24-48 hours before processing. CSF and serous fluid do not require transport media unless they are very cellular. A small piece of fresh biopsy sample can be shaken in isotonic saline to liberate cells or a piece of tissue can be injected with cell culture medium and chopped into small pieces to release cells for flow cytometry.

Sample preparation

Prior to staining with antibody, the white cells need to be separated from the red cells. This can be done by either density gradient centrifugation or whole blood lysis. The

advantage of whole blood lysis is that it minimises the loss of white cell fractions and thus permits subsequent quantification of lymphocytes subsets. Whole blood lysis is the method we used to prepare the PB and BM samples for freezing prior to molecular analysis. This is the method described in steps 6-12 of Appendix 2. Following step 12 the white cell count of the sample is measured. 5 x 10^5 cells are required and this is calculated from the following formula: required volume (μl) = 500/WCC (10^9/l). The appropriate amount of lysed sample is then conjugated with the extended B-panel of fluorochrome conjugated antibodies:

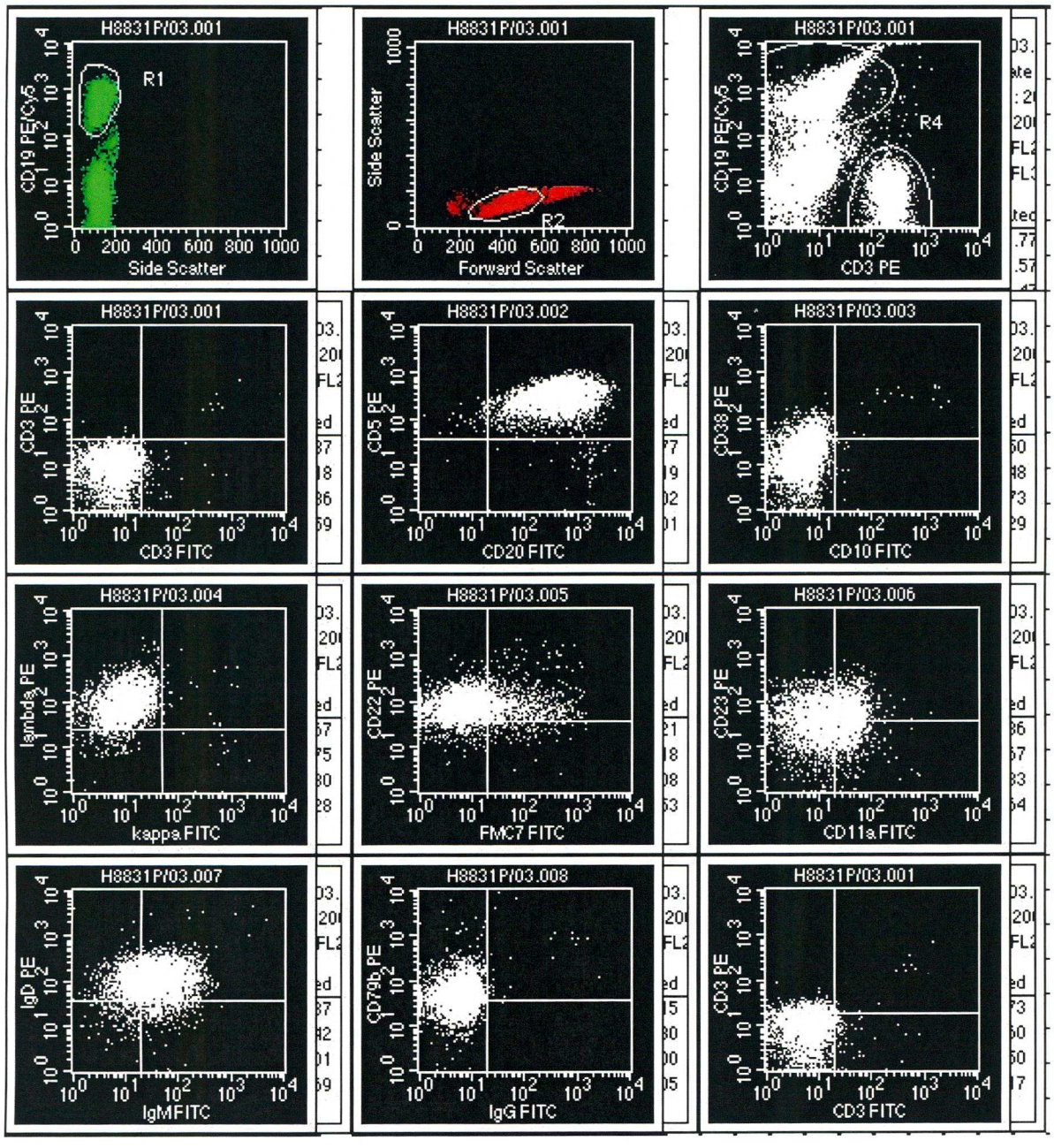
CD3/CD3/CD19
CD10/CD38/CD19
FMC7/CD22/CD19
IgM/IgD/CD19
CD103/CD25/CD19
CD38/CD52/CD19
CD20/CD5/CD19
Kappa/Lambda/CD19
CD11a/CD23/CD19
IgG/CD79b/CD19
CD11c/CD62L/CD19

The fluorochromes used are fluorescein isothiocyanate (FITC), phycoerythrin (PE) and Cy5. These differ in their emission spectrum so it is possible to analyse cells labelled with three different antibodies at once against their size (forward scatter) and granularity (side scatter).

Analysis

First a scattergram is produced of forward scatter versus side scatter. Then the population of cells of interest is identified and drawn around (gated). The fluorescent data for the cells that are within this area, or gate, can then be assessed on a histogram – this represents the surface antibody expression of the cells. (Figure A1.1)

Figure A1.1. Extended B-panel flow cytometry analysis document



Immunocytochemistry

Immunoperoxidase and alkaline phosphatase anti-alkaline phosphatase are the 2 commonly used immunocytochemistry techniques (Matutes *et al*, 2001). The former is simpler and is particularly useful for the study of lymphoid cells, whereas the latter permits good preservation of cell morphology. Immunoperoxidase can be performed with directly labelled antibodies or via an indirect technique involving several layers (described below). Unstained blood and marrow slides can be stored at minus 20°C until needed.

Cytocentrifuge slides are allowed to dry for at least 6-8 hours (if fresh) or thawed (if frozen) and the area to be studied marked. The slides are fixed in acetone, allowed to dry and the area marked with a silicone ring. The slides are then incubated with the first layer of monoclonal antibody, washed and immediately incubated with the second layer of monoclonal antibody. The slides are washed twice, incubated with the third layer of antibody and labelled with peroxidase. The slides are again washed twice and then incubated with a diaminobenzidine solution, rinsed and counterstained prior to a final wash. The slides are then allowed to dry prior to microscopy.

Immunohistochemistry

Fresh slides of paraffin embedded tissue are made and treated to allow antibody penetration. As above, both direct and indirect methods of antibody staining are feasible, although the indirect method is more commonly used as it is more sensitive. In the indirect method an unlabeled primary antibody reacts with tissue antigen and then a secondary antibody, labeled with a fluorescent dye or an enzyme, is added and this reacts with the first unlabeled antibody. A biotinylated secondary antibody is commonly coupled with streptavidin-horseradish peroxidase and then reacted with diaminobenzidine to produce a brown stain wherever primary and secondary antibodies are attached.

Molecular techniques

Conventional Cytogenetics

For conventional cytogenetics a fresh sample of involved blood, marrow or tissue, in a preservative free heparin medium, must get to the laboratory within 24 hours of being taken. $1x10^6/l$ cells are cultured and then colchicine is added which arrests cell division at mitosis. The cells are put into a hypotonic solution and then fixed and spread onto slides prior to banding and analysis.

FISH

FISH can be carried out on thin sections if only a small amount of tissue is available. Alternatively, if more tissue is available, thick sections can be cut and nuclei extracted. Slides are prepared and then fixed. Thin sections then require enzyme digestion to allow the probe access to the target DNA. The slides are then dehydrated sequentially in progressively stronger concentrations of ethanol. The slides are then incubated with formamide hybridisation buffer prior to probe application. They are then allowed to hybridise and subsequently washed. This stage is light sensitive and timing of the washes is critical. The slides are then counterstained and read with an imaging microscope.

PCR

PCR allows amplification of specific DNA sequences. Short DNA primer sequences, complementary to the ends of the sequence of interest, are used. These are mixed with the sample DNA along with nucleotides and a thermostable DNA polymerase. A thermocycler is used to heat and cool the mixture through many programmed cycles. Cycles consist of a denaturation stage where the two strands of DNA come apart at a very high temperature (~93°C), the mixture is then cooled slightly (50-60°C) to allow the primers to bind and then reheated (~73°C) to allow strand extension. This results in four copies from the original two. This process can be repeated many times. The end product is then separated out by electrophoresis on an ethidium bromide containing gel and read under ultraviolet light.

Appendix 2: Processing samples for LPD project

1. Per patient need: 4 BMA slides, 4 PB slides, 4mls PB in EDTA, 2mls PB in plain/serum tube (once sample taken stand tube in rack if possible), 2mls BMA in EDTA, 4 pieces parafilm, 3 12ml tubes with caps, 4 eppendorfs with lids, 80mls phosphate buffered solution (PBS), 40mls ammonium chloride (NH_4Cl), 6 pastettes, trephine in formalin pot with completed HMDS form.
   (1l PBS = 8.5g NaCl + 0.382g diNaHPO$_4$ + 0.462g KdiHPO$_4$ + 1l distilled water; 1l NH_4Cl 0.86% = 8.6g NH_4Cl + 1l distilled water)
2. Also need: centrifuge for 12ml tubes, permanent marker, waterbath at 37°C.
3. Wrap slides tightly in parafilm (stretch first) to ensure no air bubbles are trapped. Put 2 slides back to back per piece of parafilm. If wearing gloves try to avoid getting powder on slides or parafilm. Label outside of parafilm with patient's name number and 'PB'/'BM'.
4. Pastette serum into eppendorf with white/orange lid and label both tube and lid (with patient name, 'serum' and number as per David's list).
5. Label 12ml tubes (patient number (as above) plus 'PCR'/'BM'/'PB') and set up racks.
6. Using pastette put 2mls PB in 'PB' and 'PCR' tube. With clean pastette put 2mls BMA in 'BM' tube. (Generally use all the sample.)
7. Add 10mls NH_4Cl to 'PB' and 'BM' tubes. Put in waterbath at 37°C for 5 minutes. Add 10mls PBS to 'PCR' tube. Mix well. Cap tube. Centrifuge all tubes at 2000rpm for 4minutes. Remove cap. Decant supernatant. Flick 'PB' and 'BM' tubes to break up pellet. Take care with 'PCR' tube not to pour off sample.
8. Add 10mls NH_4Cl to 'PB' and 'BM' tubes. Put in waterbath at 37°C for 5 minutes. Add 10mls PBS to 'PCR' tube. Mix well. Cap tube. Centrifuge at 2000rpm for 4minutes. Remove cap. Decant supernatant (Care with 'PCR' tube). Flick 'PB' and 'BM' tubes to break up pellet.
9. Using clean pastette transfer sample from 'PCR' tube to eppendorf with yellow lid and label both eppendorf tube and lid (with patient name, number and 'PCR').
10. Add 10mls PBS to the 'PB' and 'BM' tubes. Mix. Recap tube. Centrifuge at 2000rpm for 4minutes. Remove cap. Decant supernatant. Flick tubes.

11. Add 10mls PBS. Mix. Recap tube. Centrifuge at 2000rpm for 4minutes. Remove cap. Decant supernatant. Flick tubes.
12. Add 10mls PBS. Mix. Recap tube. Centrifuge at 2000rpm for 4minutes. Remove cap. Decant supernatant.
13. Using clean pastettes transfer samples from 12ml tubes to eppendorfs with red (PB) and green (BM) lids and label both eppendorf tube and lid (with patient name, number and 'PB'/'BM' respectively).
14. Put all samples in -20°C freezer.

NB - PBS should be stored in the fridge and discarded after 4 weeks.

Appendix 3: The WHO classification of Lymphoid Neoplasms (Jaffe *et al*, 2001)

B-CELL NEOPLASMS

Precursor B-cell neoplasm

Precursor B lymphoblastic leukaemia/lymphoma

Mature B-cell neoplasm

Chronic lymphocytic leukaemia/small lymphocytic lymphoma

B-cell prolymphocytic leukaemia

Lymphoplasmacytic lymphoma

Splenic marginal zone lymphoma

Hairy cell leukaemia

Plasma cell myeloma

Solitary plasmacytoma of bone

Extraosseous plasmacytoma

Primary amyloidosis

Heavy chain disease

Extranodal marginal zone B-cell lymphoma of Mucosa-Associated Lymphoid Tissue

Nodal marginal zone B-cell lymphoma

Follicular lymphoma

Mantle cell lymphoma

Diffuse large B-cell lymphoma

Mediastinal (thymic) large B-cell lymphoma

Intravascular large B-cell lymphoma

Primary effusion lymphoma

Burkitt lymphoma/leukaemia

B-cell proliferations of uncertain malignant potential

Lymphomatoid granulomatosis

Post-transplant lymphoproliferative disorder, polymorphic

T-CELL AND NK-CELL NEOPLASMS

Precursor T-cell neoplasms

Precursor T lymphoblastic leukaemia/lymphoma

Blastic NK cell lymphoma

Mature T-cell and NK-cell neoplasms

T-cell prolymphocytic leukaemia

T-cell large granular lymphocytic leukaemia

Aggressive NK cell leukaemia

Adult T-cell leukaemia/lymphoma

Extranodal NK/T cell lymphoma, nasal type

Enteropathy-type T-cell lymphoma

Hepatosplenic T-cell lymphoma

Subcutaneous panniculitis-like T-cell lymphoma

Mycosis fungoides

Sezary syndrome

Primary cutaneous anaplastic large cell lymphoma

Peripheral T-cell lymphoma, unspecified

Angioimmunoblastic T-cell lymphoma

Anaplastic large cell lymphoma

T-cell proliferation of uncertain malignant potential

Lymphomatoid papulosis

HODGKIN LYMPHOMA

Nodular lymphocyte predominant Hodgkin lymphoma

Classical Hodgkin lymphoma:

- Nodular sclerosis classical Hodgkin lymphoma
- Lymphocyte-rich classical Hodgkin lymphoma
- Mixed cellularity classical Hodgkin lymphoma
- Lymphocyte-depleted classical Hodgkin lymphoma

Appendix 4: KATH consent form

Name:
Hospital Number:

Consent form for patients being investigated for possible lymphoproliferative disorders (LPDs)

Hello. My name is Dr Liz Stephens and I work at Komfo Anokye Teaching Hospital. I would like to talk to you about a study we are doing to find out what LPDs people who live in Ghana get.

LPD is a disease that can cause lumps to appear on or in your body and can affect blood, inside bones, glands and other areas of the body. You have been referred to this clinic because your doctors think you may have a LPD.

In order to find out if you have a LPD you will need to have some tests including a bone marrow test and blood tests. You may also need some x-rays or scans and possibly a piece of the lump may need to be removed for tests. Some of the procedures are uncomfortable (e.g. a blood test) and some are more painful (e.g. a bone marrow test). Local anaesthetic (where you are awake, but the pain is numbed) is used for the bone marrow test and most of the lymph node biopsies, a general anaesthetic (where you are asleep) is used for some of the lymph node biopsies.

If you take part in the study extra tests will be done on samples taken. Some of these tests will be done in Ghana, some will be done in Britain. You will not have to undergo any extra investigations as part of the study.

There is no extra cost for being part of the study, but you will get extra results if you take part. These results will help your doctors decide which treatment is best for you. You will be told any extra results at your clinic visits.

If you do not want to take part in this study you are free to refuse and it will not affect how you are looked after by your doctors. You are also free to withdraw from the study at any time.

Signed/thumbprint.....Date.....
(parent or guardian for children under 16 years)

Signature/thumbprint of child if under 16 years.....

Witnessed by.....Position.....
(For participants who do not speak English this form will be translated into their local language)

Appendix 5: KBTH consent form Name:
Hosp no:

INFORMED CONSENT TO PARTICIPATE IN RESEARCH

Principle Investigator:
Dr Elizabeth Stephens
Department of Medicine
KATH PO Box 1934
Kumasi

E-mail lizstephens@doctors.org.uk
Telephone 0243454800

Co-Investigators:
Dr Imelda Bates
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool L3 5OA

Dr Roger Owen
HMDS, Leeds General Infirmary
Great George Street
Leeds

Dr George Bedu-Addo
School of Medical Sciences
KNUST, Kumasi

Dr Bartholomew Akanmori
Noguchi Memorial Institute
Accra

Dr Ivy Ekem
Department of Haematology
University of Ghana Medical School
Accra

E-mail ivyekem@ug.edu.gh
Telephone 673036 Ext 5363

Title of project: Lymphoproliferative disorders (LPDs) in Ghana

You are being asked to volunteer as a participant in a research study. This form is designed to provide you with information about this study and to answer any of your questions. After going through this form you may refuse to participate in the study. This will not affect your management in any way.

PURPOSE OF STUDY: This study has been designed by Ghanaian and British doctors and will be carried out by them. The aim of the study is to discover more about lymphoproliferative disorders in Ghana, in particular, which lymphoproliferative disorders occur in Ghana and whether they are different to the lymphoproliferative disorders that are seen in other countries.

PROCEDURES: If you decide to participate in the study, you will have your clinical history taken (how the disease started and progressed) and will have a full physical examination. These details will be recorded on a questionnaire. Your doctor will decide which investigations you need to diagnose your LPD. If you have any blood tests, x-rays or scans, the results of these will be noted on the questionnaire. If your doctor decides you need a bone marrow test and/or lymph node biopsy, part of this sample will undergo extra investigations. An extra ten millilitres (about two teaspoonfuls) of blood will be taken for extra tests that look at exposure to malaria and presence of LPD cells or extra proteins in your blood.

BENEFITS: This study will tell us more about whether you have a LPD and if so what type. This will help guide your doctor when deciding what treatment to give you. The study will also tell us more about what types of LPD occur in Ghana. This may help us to diagnose and treat patients with LPD better in the future.

RISKS: The only extra procedure that may be performed as part of this study may be an extra blood test. Where possible we will try to take all the necessary blood tests for the study when you are having your routine LPD blood tests taken. Rarely, you might experience minor bruising at the site of blood taking as with any blood test.

CONFIDENTIALITY: All information obtained on you during the course of this study will be kept confidential. Should the results of this study be published, you will be referred to only by number. Should the samples taken from you be needed for any other investigation, the investigator understands that you will be informed and your written consent obtained first.

SUBJECT RIGHTS: Any questions you have involving the research and your rights may be addressed to:

Dr Ivy Ekem
Department of Haematology
University of Ghana Medical School
Tel 673036 Ext 5363

Your participation in this study is voluntary and you are free to withdraw without penalty at any time. The treatment of your condition will not be affected. You will be given a copy of this form to keep. If you have any problems relating to the researchers, contact the Executive Secretary, University of Ghana Medical School, PO Box 4236, Accra.

Signed/thumbprint.....Date.....
(parent or guardian for children under 16 years)

Signature/thumbprint of child if under 16 years.....

Witnessed by.....Position.....Date.....
(For participants who do not speak English this form will be translated into their local language)

Investigator.....Date.....

Appendix 6: Consumables required per patient

The following consumables were required per patient entered:

8 slides for the project, an additional 3 slides per KATH patient

2 EDTA tubes and 1 serum tube

10% formalin in an eppendorf (and formalin in pot if also for lymph node biopsy)

4 pieces of parafilm

3 12ml tubes with caps

4 eppendorfs with lids

80mls PBS

40mls NH_4Cl

6 pastettes

One litre of PBS was made by mixing 8.5g NaCl and 0.382g di$NaHPO_4$ and 0.462g K$diHPO_4$ in 1 litre of distilled water. This was stored in a refrigerator for up to four weeks. One litre of 0.86% Ammonium Chloride was made by mixing 8.6g of NH_4Cl in one litre of distilled water. This was stored at room temperature indefinitely.

Appendix 7: Project Pro Forma

Pro forma for patients investigated for LPD

At presentation

Name: Sex:

Date of birth/Age: Employment:

Date of presentation to teaching hospital: Hospital:

Source of patient referral to haematology clinic: Clinician:

Presenting symptoms:

Sweats yes/no wt loss severe/mod/mild/no fevers yes/no
Performance status: no symptoms/symptoms, but ambulatory/Bedridden<half day/ bedridden>half day/chronically bedridden
Examination findings at presentation:
General:

Liver(cm): Spleen(cm): Nodes:

Medication History:

When last took herbal medications:

Frequency of malaria symptoms (per yr):

Other previous Infections:

Past medical history:

Tests booked: Bloods requested:

Scans requested:

?lymph node biopsy booked yes/no Date:

Date blood and marrow session:

When available/1st follow-up

FBC: Hb(g/dl) WCC($x10^9$/l) Plts($x10^9$/l) MCV(fl)

Diff WCC%: Neut(%) Lymph(%) MID (%)

Diff WCC count: Neut($x10^9$/l) Lymph($x10^9$/l) MID($x10^9$/l)

Blood film findings:

Biochem results: Ur/BUN(mmol/l) Creatinine(μmol/l) LDH(U/l)

Albumin(g/l) Bilirubin(μmol/l) ALT(U/l) AST(U/l)

Alk phos(U/l) Ca(μmol/l) ESR(mm/hr) CRP

HIV status known? yes/no If known - result pos/neg

If FNA performed: Site:

Date:

Result:

If biopsy taken: Site biopsied:

Date biopsied:

Results of biopsy:

Posted to HMDS – date: By whom:

If scans/x-rays performed: Type scan/x-ray:

Date of scan/x-ray:

Results of scan/x-ray:

Provisional diagnosis:

Treatment initiated:

To be filled in at blood and marrow session

Marital status: Married/Living with sexual partner/Single/Separated/Divorced/Widowed

Live: Urban/Peri-Urban/Rural

How many people are currently living in your household?

How many dependents do you have?
How many children (aged 0-5) live with you in the same house?

What is the highest level of formal education that you have had?
No formal education/Primary-incomplete/Primary-complete/JSS-incomplete
JSS-complete/SSS-incomplete/SSS-complete/University/Postgraduate/Adult education

What is your current occupation?

Managerial/professional/administrative		Skilled manual worker (driver, brick layer)	
Manual worker (house servant, waiter)		Own business - type of business	
Subsistence farmer	Trader	Unemployed	House wife
Student	Retired	Other - specify	

Main roofing material of the house you now live in?

Grass/thatched/tin	Corrugated tin roof with unfinished ceiling
Tile with unfinished ceiling	Corrugated tin roof with finished ceiling
Tile with finished ceiling	Other - specify

Does your house have any of the following:
Electricity yes/no TV yes/no Kitchen stove yes/no Refrigerator yes/no

How did you come to the hospital?
Walking/bicycle/bus/shared taxi/private taxi/tro-tro/private car/motorbike/other – specify

Who accompanied you to the hospital?

Partner/spouse	Child under 16yrs	Child over 16yrs	Sibling
Other relative – specify		Other – specify	No one

Trephine posted to HMDS – date: By whom:
Aspirate report:

Flow results:

HMDS results:

Ann Arbor stage:

Number of extranodal sites:

6 month follow-up
Treatment so far:

Current symptoms:

Current examination:

FBC: Hb	WCC	Plts	MCV
Diff WCC(%):	Neut	Lymph	Mono

Blood film findings:

Other relevant investigation results:

Repeat blood and marrow session relevant? yes/no If so date booked:

12 month follow-up
Treatment so far:

Current symptoms:

Current examination:

FBC: Hb	WCC	Plts	MCV
Diff WCC(%):	Neut	Lymph	Mono

Blood film findings:

Other relevant investigation results:

Repeat blood and marrow session relevant? yes/no If so date booked:

18 month follow-up
Treatment so far:

Current symptoms:

Current examination:

FBC: Hb	WCC	Plts	MCV
Diff WCC(%):	Neut	Lymph	Mono

Blood film findings:

Other relevant investigation results:

Repeat blood and marrow session relevant? yes/no If so date booked:

24 month follow-up
Treatment so far:

Current symptoms:

Current examination:

FBC: Hb	WCC	Plts	MCV
Diff WCC(%):	Neut	Lymph	Mono

Blood film findings:

Other relevant investigation results:

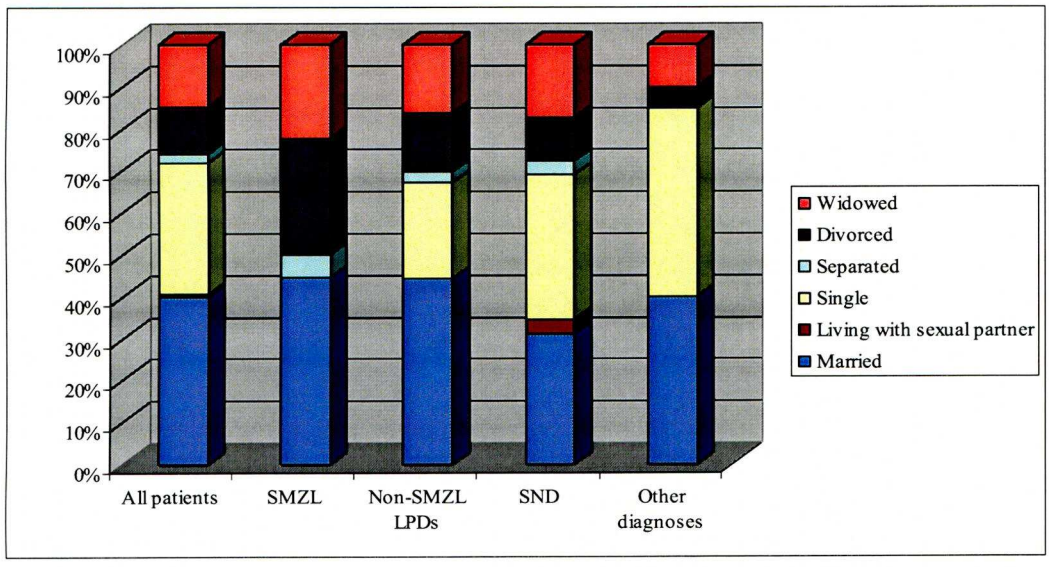
Repeat blood and marrow session relevant? yes/no If so date booked

Appendix 8: Supplementary socioeconomic data

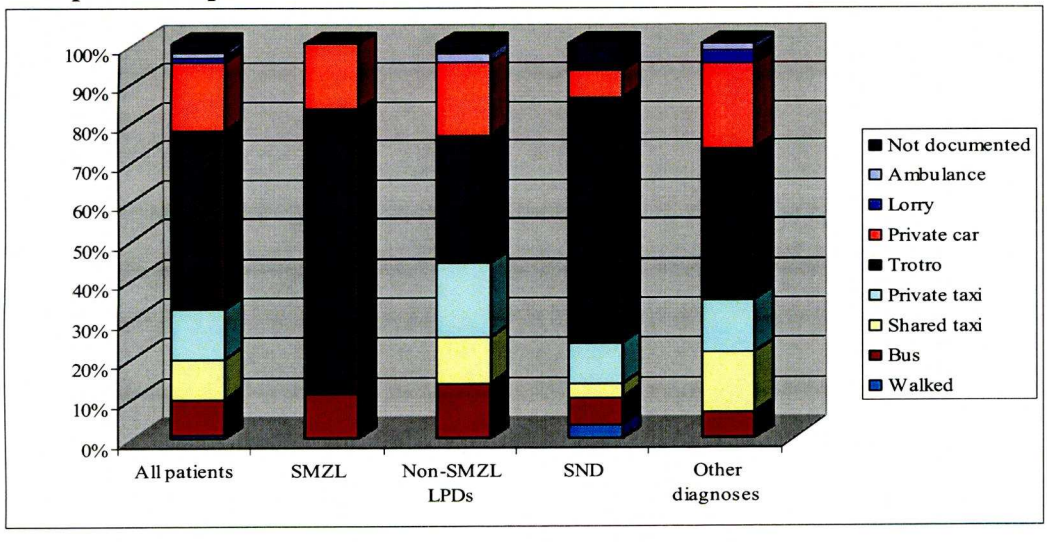
Co-habitees and dependents

	All patients n=150	SMZL n=18	Non-SMZL LPDs n=43	SND n=29	Other diagnoses n=60	p
Number of other people living in house median (range)	6.5 (0-50)	10 (3-17)	6 (0-20)	7 (1-20)	7 (1-50)	0.202 KW
Number of dependents median (range)	0 (0-30)	2 (0-10)	1 (0-30)	0 (0-15)	0 (0-10)	0.247 KW

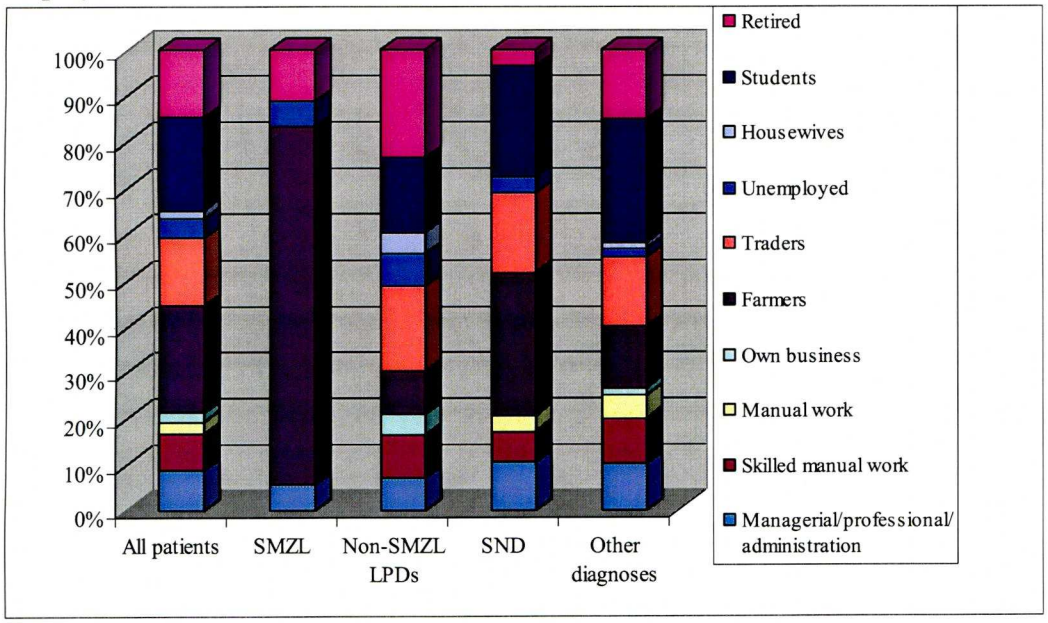
Marital status



Transport to hospital



Employment


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
All patients
SMZL
Non-SMZL LPDs
SND
Other diagnoses
Retired
Students
Housewives
Unemployed
Traders
Farmers
Own business
Manual work
Skilled manual work
Managerial/professional/ administration

Appendix 9: Ethical principles and benchmarks for multinational research (Emanuel *et al*, 2004)

Principles	Benchmarks
Collaborative partnership	Develop partnerships with researchers, makers of health policies, and the community. Involve partners in sharing responsibilities for determining the importance of health problem, assessing the value of research, planning, conducting, and overseeing research, and integrating research into the health-care system. Respect the community's values, culture, traditions, and social practices. Develop the capacity for researchers, makers of health policies, and the community to become full and equal partners in the research enterprise. Ensure that recruited participants and communities receive benefits from the conduct and results of research. Share fairly financial and other rewards of the research.
Social value	Specify the beneficiaries of the research—who. Assess the importance of the health problems being investigated and the prospective value of the research for each of the beneficiaries—what. Enhance the value of the research for each of the beneficiaries through dissemination of knowledge, product development, long-term research collaboration, and/or health system improvements. Prevent supplanting the extant health system infrastructure and services.
Scientific validity	Ensure that the scientific design of the research realizes social value for the primary beneficiaries of the research. Ensure that the scientific design realizes the scientific objectives while guaranteeing research participants the health-care interventions to which they are entitled. Ensure that the research study is feasible within the social, political, and cultural context or with sustainable improvements in the local health-care and physical infrastructure.

Ethical principles and benchmarks for multinational research continued

Principles	Benchmarks
Fair selection of study population	Select the study population to ensure scientific validity of the research. Select the study population to minimize the risks of the research and enhance other principles, especially collaborative partnership and social value. Identify and protect vulnerable populations.
Favourable risk-benefit ratio	Assess the potential risks and benefits of the research to the study population in the context of its health risks. Assess the risk-benefit ratio by comparing the net risks of the research project with the potential benefits derived from collaborative partnership, social value, and respect for study populations.
Independent review	Ensure public accountability through reviews mandated by laws and regulations. Ensure public accountability through transparency and reviews by other international and nongovernmental bodies, as appropriate. Ensure independence and competence of the reviews.
Informed consent	Involve the community in establishing recruitment procedures and incentives. Disclose information in culturally and linguistically appropriate formats. Implement supplementary community and familial consent procedures where culturally appropriate. Obtain consent in culturally and linguistically appropriate formats. Ensure the freedom to refuse or withdraw.

Ethical principles and benchmarks for multinational research continued

Principles	Benchmarks
Respect for recruited participants and study communities	Develop and implement procedures to protect the confidentiality of recruited and enrolled participants. Ensure that participants know they can withdraw without penalty. Provide enrolled participants with information that arises in the course of the research study. Monitor and develop interventions for medical conditions, including research-related injuries, for enrolled participants at least as good as existing local norms. Inform participants and the study community of the results of the research.